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It is characteristic of Science and Progress that they continually open new fields to our vision.—PASTEUR

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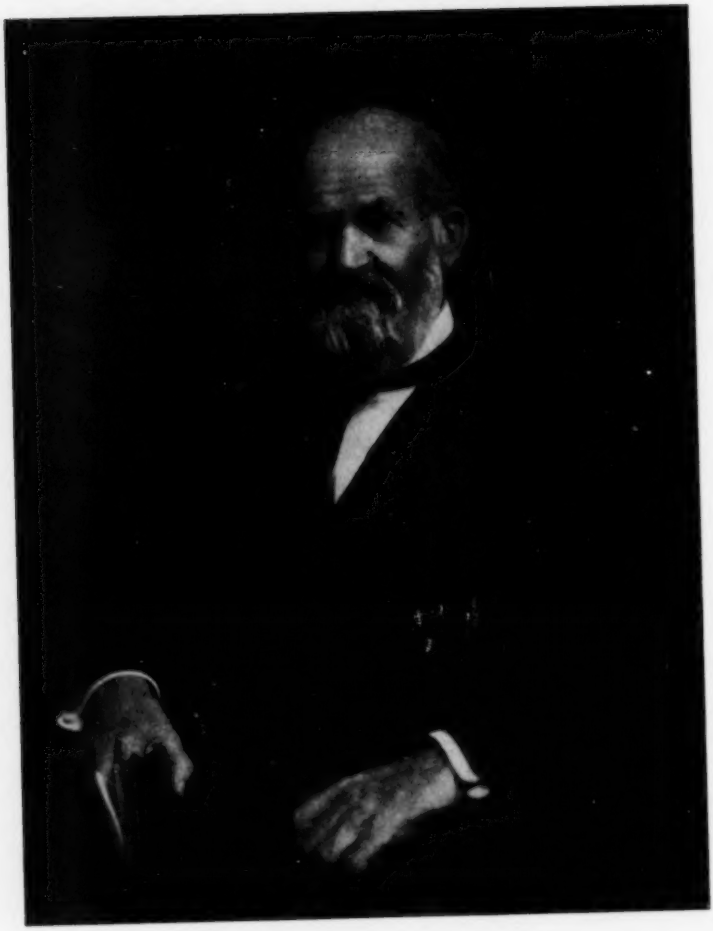

THOMAS JONATHAN BURRILL
[1839–1916]

IN MEMORIAM THOMAS J. BURRILL

ERWIN F. SMITH

In the recent death (April 14) of Prof. Thomas J. Burrill of the University of Illinois, there passed away, at a ripe old age but still in possession of all his faculties, a lovable man of uncommon personality, and one who contributed materially during his earlier years to the advancement of American science. In America we have a peculiar way of treating all those who have demonstrated the possession of research ability of a high order, which may be designated as a method of extinction by promotion. As soon as a man becomes conspicuous through his researches, boards of control find other things for him to do, more in keeping with their ideas of efficiency and eternal fitness, and he ceases to contribute further, except perhaps very indirectly, to the advancement of science. Professor Burrill was no exception to this rule. He never lost his interest in science and having a high order of mind he was peculiarly fitted to be a productive research worker, but from middle life on it was his misfortune, recognized by him as such, but borne with cheerfulness, to have his time absorbed by administrative duties connected with his university, of which he was at one time the acting head. His actual contributions, however, were amply sufficient to perpetuate his memory.

In addition to his mycological studies, which he pursued with great eagerness and with good results ("Fungi of Illinois") he studied the bacteria at a time (1870–1882) when literature was scanty, methods were crude, and microscopes were not what they are today. Into this field of darkness, or at best of dim half-lights and perplexed gropings, which he has described to me in memorable words, Burrill projected his keen intellect and brought forth the beginnings of a whole new science, i.e., he discovered and demonstrated in "pear blight" the first bacterial disease

of plants. To Burrill and America belongs this honor, whatever other honor belongs elsewhere! Just as Pasteur's contribution to science is more vital than Koch's, because it was earlier and was pioneer work, so Burrill's discovery was more difficult to make and hence more worthy of praise, than anything that has come after. Anyone of ordinary capacity can follow a blazed trail, but only a great man can hew a path through the unbroken wilderness to be a highway for all men who come after!

Burrill did not publish on pear blight fully, in the modern sense of that word, for he was a pioneer, but in studying the freshly diseased tissues (and he had the wisdom to select just those) he saw clearly in many sections that fungi were not there and that swarms of bacteria (called by him *Micrococcus amylovorus*) were always present and were therefore probably the cause of this mysterious disease. Acting on this assumption he took masses of these bacteria which his microscope had shown to be free from fungi (with a multitude of whose forms he was already very familiar) and with them by inoculation reproduced the pear disease, not once but many times. Others, elsewhere, in these same early days made similar announcements, but were less fortunate or less painstaking, since no one in later days has been able to confirm their findings, whereas Burrill's discoveries have been confirmed a hundred times, and relate to one of our most serious orchard diseases, known for a hundred years, and for the control of which the nation and the orchard states are still spending much time and money.

Professor Burrill was born at Pittsfield, Mass., April 25, 1839. He was educated at the Illinois State Normal School, and was always a teacher, and a good one. He held honorary degrees from the University of Chicago (Ph.D., 1881) and The Northwestern University (LL.D.,1893), and was a member of various scientific societies. I remember seeing him first at meetings of the American Association for the Advancement of Science, of which he was long a member, and this year president of Section G (Botany). He had also been president of the American Microscopical Society, 1885–86, and was president of the Society of American Bacteriologists at the time of his death.

Professor Burrill was very companionable and very helpful to his students. He was also much respected by his colleagues the country over. At one of the last scientific gatherings he attended (the twenty-fifth anniversary of the Missouri Botanic Garden held at St. Louis in 1914), when his name was incidentally mentioned by one of the speakers there was a round of applause from the crowded room. I did not see him after this time but he was then (at 75) very well preserved and intellectually keen.

The ancient Greeks had a proverb "Let not a man boast that he has had a happy life until the day of his death." Professor Burrill would not have boasted of anything since he was quiet and unassuming rather than loud and aggressive, but it may be said for him, that he represented the best type of scientific mind and now that he has gone we may say as we close the ranks and turn away: Happy was this man because he lived unobtrusively, serenely and usefully, and because he died full of years and of honor, loved by all his intimates, and respected by all who knew him.

RESOLUTIONS ADOPTED AT THE URBANA MEETING OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS IN REGARD TO THE WORK OF PROFESSOR BURRILL.

WHEREAS, It is rarely possible for a scientist to make a discovery of such fundamental importance that it serves to develop an entirely new branch of science, and

WHEREAS, One of our hosts at this time, Dr. T. J. Burrill, made such a discovery when he worked out the cause of pear blight and thus founded the science of bacterial plant pathology,

Therefore, be it resolved, that the Society of American Bacteriologists regards it as a peculiar privilege to congratulate him for his pioneer and epoch-making work, and expresses its appreciation of his vigorous enthusiastic and inspiring address of welcome.

Be it further resolved, That a copy of these resolutions be engrossed, signed by the officers of the Society, and presented to Dr. Burrill.

STUDIES ON AEROBIC SPORE-BEARING NON-PATHOGENIC[1] BACTERIA

PART I

From the Laboratory of Hygiene and Bacteriology, Johns Hopkins University

INTRODUCTION

BY W. W. FORD

One of the most important problems of modern hygiene is the identification and classification of the bacteria in our environment. Microörganisms of various kinds exist everywhere in nature and influence profoundly all sorts of substances which affect man's physical condition. This is true of food-stuffs in general and especially true of milk which is markedly altered in its chemical composition by the bacteria which multiply in it. The microörganisms in our environment are of various sorts, pigmented bacteria, spore-bearing bacteria, yeasts, moulds, etc. Some of these forms are identified without great difficulty but our knowledge of the spore-bearing bacteria is still in a state of chaos. The reason for this lack of knowledge is not far to seek. The science of bacteriology developed primarily among physicians whose interest naturally lay in the disease-producing properties of the various parasites which infect man and the animals. Non-pathogenic bacteria were of importance chiefly as laboratory contaminations to be avoided. With the development of industrial bacteriology those species were again most carefully studied which seemed to serve some distinct purpose in nature, as for example, the nitrifying bacteria of the

[1] The term "non-pathogenic" is used here in the sense of "lacking in disease-producing properties." Many spore-bearing bacteria are at times pathogenic to small animals and instances are reported in which they may produce inflammatory reactions when vegetating on mucous surfaces. The organisms here described are however in no instances capable of producing definite diseases

soil and the lactic acid bacteria in milk. In consequence the bacteria found in nature which seem to be lacking in any definite function have been largely neglected. At various times many species of spore-bearing organisms have been described and recorded in the literature and in many instances these cultures have been kept alive in laboratories both in Europe and in America. It would seem an easy task therefore to collect the spore-bearing bacteria from different institutions, make a careful study of their properties and arrive at some conclusion as to their identity and classification, just as is done with the pathogenic species. This method of solving the difficulty is open to serious objections, however, and has not thus far proved of great value. In the first place the descriptions originally given of many of these species are meager and the original cultures have not been saved. In consequence the literature of bacteriology is thickly strewn with names of spore-bearing organisms which have absolutely no meaning. The term *Bacillus subtilis* for instance is applied to almost any large microorganism which forms spores readily and grows abundantly on artificial media, and cultures identified as *Bacillus subtilis* by different bacteriologists are often found to have little or nothing in common. Again the cultures which have been kept alive have in many instances so changed in character as no longer to give the reactions orginally described. Thus Migula (1897) found that of some six hundred cultures obtained by him from the laboratories in Germany only a small number had the characteristics first ascribed to them. Finally, pure strains of spore-bearing bacteria are more difficult to keep in direct descent in the laboratory than are other species. When cultures become contaminated it frequently happens that the contaminating species is picked up from the plates made to purify the strain and carried on as the original. This has happened a number of times in our own laboratory during the past few years and in consequence we have become very sceptical of the value of any conclusions based upon a comparison of existing stock cultures.

A number of years ago an attempt was made, in the laboratory of Dr. Adami in Montreal (Ford, 1903) to separate and classify

the sporulating organisms by the use of carbohydrates. The result of this work was not entirely satisfactory because of the difficulty of establishing the fundamental species from which to build up our system of classification. During the past few years, however, a number of very valuable papers on spore-bearing bacteria have appeared in the literature and have cleared up some of the most difficult points. Of especial importance is the work of Meyer (1903) and his collaborators, Gottheil (1901) and Neide (1904) in Germany, and the work of Chester (1903) in this country. As a result of the efforts of these authors we now have accurate descriptions and definite means of identification of a small number of our most common spore-bearing species.

Some four years ago a large number of spore-bearing bacteria was obtained from raw milk and from milk heated to various temperatures from 60° to 100° and so much difficulty was encountered in their identification that it seemed as if the time was ripe for a more extensive investigation of the subject, based upon the work above referred to. The problem was first undertaken by Mr. Lawrence and myself with the organisms from milk. After a working basis had been obtained for the classification of these species a study of the spore-bearing bacteria of water was undertaken by Dr. Laubach, and of the soil by Dr. Laubach and Mr. Rice with the object of testing the classification already adopted for milk bacteria and of adding to it such species as had not previously been encountered in our work. Finally stock cultures of well-known species were obtained from the Kral collection in Vienna, the Winslow collection in the American Museum in New York, from the laboratories of hygiene of the University of Pennsylvania, and of the University of Chicago, and the bacteriological laboratory of the Sheffield Scientific School, and our cultures were compared with them. From the start of the work however, our object has been to establish clearly the different types of spore-bearing organisms in our own laboratory and then to link these types up with types already established by other observers. Altogether over 1700 cultures have been studied from various sources, milk, soil, dust,

water, intestinal contents and contaminated plates. From this number we have obtained 28 distinct types of which 22 are clearly to be identified as well known species, 2 are distinct varieties of old types and 4 are evidently new species. In general our aim has been to clarify our knowledge in regard to old species and not to establish new types except when our isolations showed certain characteristics not already referred to in the literature and of distinguishing importance.

The media employed in this work were the standard media of the laboratory. A great deal of emphasis was laid upon the reactions with gelatin, with litmus milk, with glucose, saccharose, and lactose broth, with glucose litmus agar and with Loeffler's blood serum. The morphology was studied from smears made from plain and glucose agar cultures 6 to 8 hours old and 22 to 24 hours old, and from cultures 1 to 2 weeks old, the organisms being always stained with Gentian violet. The same preparations were used later for measurements and for illustrations. The method of sporulation and the size, shape and position of the spore were observed with great care. A study of the spore wall, and its differentiation into the exine and intine of Gottheil and Chester, while interesting and important, proved of little help in classification. The method of spore-germination was likewise found relatively valueless. Nearly every type of spore-germination could eventually be found with most species and our observations were so inconstant as not to furnish any basis for classification. Micro-chemical reactions, while undoubtedly of great value, could not be worked out with any degree of thoroughness and were eventually discarded. Careful observations were made upon the thermal death points which were established with broth cultures subjected to various degrees of temperature in the Arnold sterilizer and in the autoclave. In general our classification may be said to rest upon morphological and tinctorial properties, spore-formation and cultural reactions. How valuable our results are can only be determined by the extent to which other workers may be able to utilize this classification in subsequent investigations.

SPORE-BEARING BACTERIA IN MILK

BY J. S. LAWRENCE AND W. W. FORD

It has been pointed out by a number of observers (Flügge, 1904; Ford and Pryor, 1904) that milk heated to any temperature above 60°C, if kept in a warm place, shows an excessive development of spore-bearing bacteria which are ordinarily inhibited by the lactic acid bacteria universally present. Hueppe (1884) was possibly the first to call attention to the presence of aerobic spore-bearing forms in milk but it is impossible to say now what his *Bacillus butyricus* (an aerobe) really was. Subsequently Loeffler (1887) described an organism from boiled milk which had been allowed to clot, under the name *Bacillus lactis albus*, now known as *Bacillus albolactus* Migula. Considerably later Flügge (1894) took up the question at some length and described eleven different species found in boiled milk and to them he ascribed an etiological rôle in the summer diarrhoea of infants. Several of the organisms described by Flügge are now considered identical with such common saprophytes as *Bacillus vulgatus* and *Bacillus mesentericus* while others can be identified with difficulty or not at all, as their originals have been lost. During the past three years we have worked out the morphological and cultural reactions of 250 spore-bearing bacteria obtained from raw milk and from milk subjected to various temperatures. The two most common species proved to be *Bacillus cereus* of Frankland and *Bacillus subtilis* of Cohn. In this differentiation we follow Chester who has given us a definite and clear conception of Cohn's species and has taken up at length the somewhat involved discussion concerning the two organisms. As a result of his work Chester decided that the real *Bacillus subtilis* of Cohn is one of the smallest of the spore-bearing species, forms central or slightly excentric spores which have a characteristic appearance and gives definite cultural reactions. The reactions as outlined by Chester we are able to confirm in the main but we disagree absolutely from him in his contention that this species is identical with *Bacillus vulgatus*, (*B. mesentericus vulgatus*) the old fashioned "potato bacillus."

The cultures identified by us as *Bacillus subtilis* corresponded in all particulars to a culture sent us several years ago by Chester and kept in the laboratory since then. The particular points by which *Bacillus subtilis* may be differentiated from *Bacillus vulgatus* are the development on glucose litmus agar where it forms a dry hard warty growth made up of dense masses of material clinging firmly to the medium, in which may be observed numerous blebs or blisters containing milky fluid, and on Loeffler's blood serum where a similar growth appears, often however with a distinct red color. On both glucose litmus agar and blood serum *Bacillus vulgatus* develops as a soft wrinkled friable mass easily broken and lifted from its substratum. On potato the *subtilis* differs from the *vulgatus*. The former produces at first a rather dense whitish or greyish mass often showing blebs similar to those on agar and blood serum, a distinct red line appearing in the potato a little distance from the growth, from which characteristic the name *Bacillus subtilis-ruber* is frequently employed. After 48 to 72 hours a wrinkling appears, the growth later becoming moist and homogeneous. *B. vulgatus* produces a wrinkled growth from the start, this becoming extremely abundant in 3 to 4 days and frequently assuming a decided pink color. The differences between the two species are somewhat difficult to describe but when potato cultures of the organisms are placed side by side the points of differentiation become clear and definite. In general the *subtilis* cultures are dry and hard on solid media and produce firm tenacious scums on fluids, while the *vulgatus* cultures are soft and mealy and their scums friable and easily broken. On a morphological basis it is extremely puzzling to attempt the differentiation of the two types. In general the rods of *Bacillus vulgatus* are longer and thinner than those of *Bacillus subtilis* while the spores are flatter and bulge the organism only a little if at all.

This view of *Bacillus subtilis* of Cohn and the interpretation put on Cohn's work by Chester is not entirely accepted by bacteriologists but we feel convinced of its correctness except in regard to the differentiation from *Bacillus vulgatus*

already mentioned. The organisms frequently regarded as *Bacillus subtilis* which are characterized by their greater size, their soft mealy growths on hard media, and their thick friable scums we agree with Chester in referring to the "cereus" group the principal type of which, *Bacillus cereus*, was first described by the Franklands. There are two strains of *Bacillus cereus* differentiated by their action on saccharose but it does not seem wise at the present time to divide the species. Our identification of *Bacillus cereus* rests upon Chester's work and upon cultures sent us by him several years ago. In addition a number of strains of *Bacillus cereus* have been received from American laboratories and from the Kral collection in Vienna, all of them agreeing with Chester's in their reactions and thus furnishing us a distinct type by means of which our own strains were identified. *Bacillus cereus* is the most widely distributed aerobic spore-bearing organism in nature in Baltimore and vicinity, as it seems to be in other localities, and possibly has more synonyms than any other species. With these types of *Bacillus subtilis* and *Bacillus cereus* clearly outlined the task of identifying the other spore-bearing organisms became somewhat simpler. *Bacillus vulgatus* was soon found so frequently as to enable us to recognize it without difficulty. One strain of this organism was obtained from the Winslow collection in New York. When freshly isolated the *vulgatus* is very characteristic and differs entirely from other species. The strains isolated in Baltimore were identical with the organisms found in Montreal several years ago and regarded there as *Bacillus vulgatus* and give reactions ascribed to the widely distributed "potato bacillus." *Bacillus mesentericus* (*B. mesentericus fuscus*) was recognized by its morphology and its cultural reactions. In this species we follow Chester. In one instance we obtained a stock culture of *Bacillus mesentericus* which gave the correct reactions as outlined by Chester, this culture coming from the laboratory of hygiene of the University of Pennsylvania. *Bacillus pumilus* of Gottheil we do not regard as a distinct species. *Bacillus aterrimus* (*B. mesentericus niger*) was identified by its production of a black or grey-black pigment, its cultural reactions resembling those of

B. vulgatus. Another organism producing a black pigment and evidently belonging to the mesentericus group was sent us by Winslow as *Bacillus lactis-niger*. It corresponds culturally to *Bacillus mesentericus*. It was not encountered in our work but is included here for the sake of completeness. The same holds true of the organism described originally as *Bacillus mesentericus-ruber* (properly *B. globigii*) a culture of which was obtained from the Kral collection in Vienna. Evidently this is an extremely rare organism in this country as it was never obtained in Baltimore either from milk or from any other source.

One of the most difficult organisms to identify was a species frequently isolated in Baltimore from milk which after boiling clots and peptonizes. In morphology and in its chief cultural reactions it corresponds closely to *Bacillus cereus* but is differentiated from this species by its acid fermentation of lactose and its coagulation of milk. This organism was evidently first described by Loeffler in 1887 as *Bacillus lactis albus* (*Bacillus albolactus* Migula). We have been unable to obtain a culture of Loeffler's organism but in his original description Loeffler differentiates this species clearly from several other organisms found in milk particularly the ones now known as *Bacillus vulgatus* of Flügge, *Bacillus liodermos* of Flügge, and *Bacillus butyricus* of Hueppe. Since Loeffler was the first to call attention to the presence of an organism in boiled milk which acidifies and clots it and which he differentiated from other spore-bearing bacteria, we feel that similar organisms from boiled milk which correspond to Loeffler's description should be regarded as identical with his species. We therefore propose to utilize the name *Bacillus albolactus* Migula, (synonym *Bacillus lactis albus* Loeffler) for the organisms isolated from the source studied by Loeffler. This organism is undoubtedly isolated from time to time by other bacteriologists and must exist in a number of laboratories. It was apparently described recently by Neide (1904) in Meyer's laboratory as *Bacillus teres*. *Bacillus albolactus* is, we believe, the common cause of the clotting and peptonization occasionally seen with boiled milk. It is undoubtedly also a contributing

factor to the changes seen in milk pasteurized at lower temperatures, 60° to 65°C., which subsequently develops a bitter taste.

Bacillus mycoides was identified without difficulty by the classical descriptions and by the work of Chester whose conclusions were based upon a culture which was sent him from our laboratory several years ago. The felted growths in the depths of agar are very characteristic and are given by but one other species, *Bacillus ramosus-liquefaciens* of Prausnitz.

For a long time we were in doubt as to the identification of the very large microörganisms which are placed in a heterogeneous group and sometimes called *Bacillus megatherium*, sometimes *Bacillus petasites*, and sometimes *Bacillus tumescens*. The first member of this group was described by De Bary (1884, 1887) whose illustrations are very characteristic. Many of the cultures identified and sent to us as *Bacillus megatherium* differed radically from De Bary's description, and Chester's conclusions in regard to the ill-defined character of the group seemed to be entirely justified. These large organisms were very abundant however and soon resolved themselves into two distinct types. One type agreed with De Bary's original description in all essential particulars and this type agreed also with an isolation of *Bacillus megatherium* by Kellermann sent us from the Winslow collection. Two cultures of *Bacillus tumescens* of Zopf agreed closely with this *Bacillus megatherium* and there seems to be no reason to regard it as a distinct species. The other type has almost the same morphology and the same cultural reactions as *Bacillus megatherium* but produces an intense yellow pigment. This type corresponds to the organism recently described as *Bacillus petasites* by Gottheil. All the organisms thus far encountered with the morphology referred to can thus easily be divided into these two main forms. The *Bacillus graveolens* of Gottheil, not the *Bacillus graveolens* of Bordoni-Uffreduzzi, seems to be merely a strain of *Bacillus megatherium* in which the bacilli have a peculiar property of growing in short spirals. It has not been encountered in our work.

The *Simplex-cohaerens* group of Chester proved possibly the

most difficult of all to clarify. Two organisms were originally described by Gottheil as distinct species, regarded by Chester however as practically identical. Strains of *Bacillus simplex* and *Bacillus cohaerens* received by us from Kral were quite different morphologically and while it is evident that we lack many of those pronounced cultural and morphological reactions which render species and groups easy to recognize yet we must not therefore place organisms together which are clearly different. On one occasion we found in milk an organism evidently identical with the strain of *Bacillus cohaerens* received from Kral and corresponding to Gottheil's original description. Subsequently this species was found five times by Dr. Laubach in soil. These organisms gave us a fairly clear idea of the species and its differentiation from *Bacillus simplex* whose description we also give here. This latter description while made from a strain isolated by Gottheil, applies also to a species subsequently found in dust by Dr. Laubach. On two occasions we isolated from milk the species described as *Bacillus fusiformis* by Gottheil. Our isolations were identical with Gottheil's in every particular. Finally on one occasion we obtained a strain with properties practically the same as those of the species described by Flügge as No. XII and now known as *Bacillus terminalis* Migula.

The 250 cultures studied were from 68 samples of milk, 12 of raw milk, 12 of milk pasteurized at 60°C., 32 of milk heated to 85°C., and 12 of boiled milk. These cultures may thus be held to represent so many various conditions in the development of the bacteria of milk as to give an accurate idea of the spore-bearing organisms of milk in Baltimore and they probably represent conditions met with elsewhere. By their combined development in heated milk they give rise to the putrid decomposition so frequently observed. As can be seen from their cultural reactions these organisms are in the majority of instances energetic protein-splitters and in practically every case rapidly dissolve the casein in milk either before or after a preliminary coagulation.

After the various types of spore-bearing organisms were established by the study of 250 cultures from the 68 samples

mentioned above another series of milks, also subjected to various treatments, was investigated with the object of testing the preliminary classification adopted. In this second portion of our work the types previously established were abundantly confirmed but no new organisms were isolated. We believe therefore that the organisms first worked out represent the spore-bearing organisms usually present in Baltimore milk. Our original 250 cultures show the various species in the following proportions.

Baltimore Milk

Bacillus cereus Frankland	124
Bacillus subtilis (Ehrenberg) Cohn	79
Bacillus albolactus Migula	25
Bacillus vulgatus (Flügge) Trevisan (*Bacillus mesentericus vulgatus* Flügge.)	15
Bacillus mesentericus (Flügge) Migula (*Bacillus mesentericus fuscus* Flügge.)	2
Bacillus fusiformis Gottheil	2
Bacillus petasites Gottheil	1
Bacillus cohaerens Gottheil	1
Bacillus terminalis Migula	1

In addition to these the following species were isolated from other sources during the work on milk and made the basis for comparison. We have reason to believe that they may occur in milk, partly from the work of others and partly because they are not infrequent in milk products. We introduce them here for completeness.

Bacillus mycoides Flügge.

Bacillus megatherium De Bary.

Bacillus simplex Gottheil.

Bacillus aterrimus Lehmann & Neumann (*Bacillus mesentericus niger* Lunt).

Bacillus niger Migula (*Bacillus lactis niger* Gorini).

Bacillus globigii Migula. (*Bacillus mesentericus ruber* Globig).

Finally a brief note is added in regard to certain other cul-

tures sent us which we do not regard as entitled to specific rank, namely

Bacil'us pumilus Gottheil.
Bacillus graveolens Gottheil.
Bacillus tumescens Zopf.

Bacillus cereus Frankland 1887

This organism was first described by the Franklands in 1887 (Franklands, 1887). It has since been described under a host of names and it is impossible to say how many different species are identical with it. It fs the most widely distributed organism of this group in Baltimore, being found abundantly in milk, soil, dust, water, and in the intestinal contents. It is particularly common as a laboratory contamination. The present description applies to cultures received from the Kral collection, from the American Museum, and from a number of American laboratories and to over a hundred of our own isolations.

Morphology. Regular bacilli with homogeneous protoplasm and rounded ends, in young cultures measuring about 0.75 by 2.25 to 4 microns. Many of the organisms show peculiar refractile bodies of various sizes as the cultures get older, presenting a characteristic appearance. The nature of these bodies is not clear as they do not give reactions for starch or volutin. They can usually be differentiated from the beginning spores. On glucose agar the bacilli are thicker and longer measuring 0.75 to 1 by 3 to 6 microns. Here the entire protoplasm of the organism is converted into the bodies mentioned above. They are globular, highly refractile, and are often as thick as the organism. (Figures 25, 26 and 27.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore-formation. Spores are formed early on both plain and glucose agar, often appearing within 24 hours or even in less time. They may be central in position, excentric or even subterminal but the latter location of the spore is rare. The spores are usually wider than the organisms from which they spring

and thus bulge the rods slightly. The free spores retain their protoplasm at the ends for some time, usually in equal amounts. Often, however, the protoplasm is greater at one end than at the other and the spore then has a characteristic appearance like an enlarged mesentericus spore. The free spores are cylindrical, soon shed their protoplasm and measure 0.5 to 0.75 by 1.125 to 1.5 microns.

Agar slant. Abundant, thick, white mealy growth along the line of inoculation sometimes with arborescent edges. In older cultures the growth is much thicker, yellowish white and may show pellucid areas surrounded by more highly refractive patches.

Agar stab. Little growth along line of inoculation but luxuriant surface growth spreading over entire surface of agar and extending to the walls of the tube.

Agar colonies. Round, raised, dense, highly refractive surface colonies. If slight amount of water of condensation be present the colonies may be amoeboid. Under low power the colonies consist of dense central nuclei with spreading peripheries made up of numerous curling and parallel chains. The colonies are soft and easily lifted from the agar. Deep colonies punctiform, stellate or rhizoid. Under the low power they are fuzzy, irregular and may resemble a chestnut burr.

Litmus glucose agar slant. Thick, yellowish-white growth along the line of inoculation and spreading out over entire surface. The medium is acidified and the growth is sometimes distinctly yellow. Typical cultures rapidly decolorize the litmus and then become alkaline and the agar turns deep blue. Occasionally the cultures are less active alkali-producers and the medium remains permanently acid. Such cultures, however, can usually be stimulated to alkali production by plating and they then give characteristic growths.

Glucose agar colonies. Surface colonies round or bizarre, heaped up, with irregular margins, smaller than plain agar colonies. Under low power granular, with dense central nuclei and irregular margins, showing fine parallel strands. Deep colonies small irregular or round. Under low power they consist of dense central nuclei with fine, irregular or parallel strands in the periphery.

Gelatin stab. Uniform growth along entire line of inoculation with a liquefaction also along entire line. The liquefaction becomes cup-shaped or sacculated with a surface scum. It is rapid and frequently in two days the entire gelatin tube is liquefied.

Gelatin colonies. Loosely filamentous colonies with dense, central nuclei and spreading irregular margins, often very thin, edges entire. Gelatin liquefied rapidly.

Broth. Very turbid in 24 hours with no scum except occasionally a slight ring growth. In two days a heavy friable scum is produced which is entirely precipitated within a short time. The medium gradually clears while a heavy flocculent precipitate is deposited.

Peptone. Very turbid in 24 hours. Scum appears usually on the second day and is soon precipitated. It is like that produced in broth but is more friable.

Potato. Thick, white, mealy growth in a few days becoming yellowish or brown with a discoloration of the potato. This brownish growth may become very moist and slimy and is occasionally measley but never vermiform. It never assumes an appearance similar to that seen with cultures of *Bacillus subtilis* or *Bacillus vulgatus.*

Litmus milk. With the majority of cultures peptonization begins immediately and progresses rapidly in three zones. Surface zone is amber, middle zone violet, the lowest zone blue. Peptonization continues until the entire milk tube is converted into an amber fluid with a slight sediment. Milk does not coagulate. With some cultures the three-zone appearance does not show but the milk is gradually changed to a muddy gray-colored fluid. Eventually, however, the same clear amber-colored fluid is produced.

Blood serum. Thick, white, dry, smooth growth. No liquefaction.

Fermentation tubes. Glucose. Abundant growth in bowl and arm. Friable scum forms which is soon precipitated. Turbidity gradually disappears and a flocculent precipitate is deposited. Reaction highly acid.

Saccharose. Abundant growth in bowl and arm. Filmy and

friable scum forms which is soon precipitated. In many cultures the reaction is acid. Reaction alkaline in the majority of cultures.

Lactose. Turbidity in bowl with scum formation. Arm clear. Reaction alkaline.

Thermal death point. The spores survive steaming one hour in the Arnold sterilizer and autoclaving at 19 pounds pressure. Killed by 20 pounds pressure.

Bacillus albolactus Migula 1900

This organism was apparently first obtained in pure culture in 1887 by Loeffler who found it in boiled milk which had soured and clotted and who named it *Bacillus lactis-albus*. It is possibly identical with *Bacillus corrugatus* Migula (1900) (Bacillus No. II Flügge), with *Bacillus bernensis* Lehmann and Neumann, (1901) and with the organism described recently by Neide as *Bacillus teres* which was also obtained from boiled milk which had subsequently soured. It is common in boiled milk in Baltimore and produces the souring, clotting, and subsequent peptonization seen so frequently in this material.

Morphology. These organisms are identical morphologically with *Bacillus cereus*. In young cultures 6 to 24 hours old, on plain agar, they have round ends and measure 0.5 to 0.75 by 2.25 to 4 microns. The protoplasm may be homogeneous or may show globular bodies of various dimensions. On glucose agar the globular bodies are much more abundant and give the organism a characteristic appearance. Here the rods measure 0.75 to 1 by 2.5 to 4 microns. (Figs. 28, 29, and 30.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed readily on plain and on glucose agar. They are abundant in 24 to 48 hours and have the same appearance as the spores of *Bacillus cereus*. They are usually central or slightly excentric and a trifle wider than the organisms from which they spring thus bulging the rods somewhat. The free spores may retain equal or unequal bits of

protoplasm at the ends and thus have a characteristic appearance. They are oval to cylindrical and measure 0.5 to 0.75 by 1.5 to 2.125 microns. The spores are frequently seen in pairs attached by their protoplasmic remnants, and also sometimes in chains.

Agar slant. Luxuriant, thick, white growth with a smooth and glistening surface, spreading over the entire surface of the agar. Some cultures show a delicate transverse wrinkling.

Agar stab. Fine, slightly arborescent growth along line of inoculation. Thick, white, wrinkled surface growth.

Agar colonies. Surface colonies thick, raised, round or bizarre, frequently showing dense, central nuclei. Under low power granular with dense, central nuclei and spreading peripheries made up of curved parallel strands. Deep colonies small, round or irregular. Under low power irregular, mossy, with irregular fuzzy margins.

Litmus glucose agar slant. Thick, yellowish-white, moist growth, spreading over the entire agar and wrinkling slightly at the base when the culture is very active. Reaction in medium acid in first few days but gradually alkali is produced and the agar turns dark blue.

Litmus glucose agar colonies. Surface colonies thin, translucent, somewhat smaller than plain agar colonies. Under low power granular with thin peripheries made up of curling parallel strands. Deep colonies round or irregular. Under low power irregular, mossy with irregular, fuzzy margins. Medium first acidified and then made alkaline.

Gelatin colonies. Surface colonies round, spreading concentrically and composed of a central loose mass of filaments denser than the surrounding zone. Deep colonies are composed of spherical masses of loose filaments with irregular, mossy or bristling margins. Rapid iquefaction.

Gelatin stab. Growth along line of puncture with a rapid cup-shaped liquefaction and scum production.

Broth. Turbidity with ring growth in 24 hours and scum formation in 2 to 3 days. Scum quickly precipitated.

Peptone. Turbidity with scum formation on the second day. Scum usually persists.

Potato. Thick, white, moist growth later becoming yellowish brown. Never wrinkled or vermiform, rarely measley. Medium discolored.

Litmus milk. Acid production and coagulation, usually within 24 hours. The coagulum is at first firm but gradually undergoes peptonization, and is usually completely dissolved at the end of three weeks. Odor distinctly faecal and very disagreeable, with a suggestion of indol.

Blood serum. Thick, white growth. No liquefaction.

Fermentation tubes. Glucose. Abundant growth in bowl extending up into closed arm which becomes turbid. Flocculent precipitate forms but usually no scum. Reaction highly acid.

Saccharose. Turbidity in bulb extending up into the closed arm. Flocculent precipitate. No scum. Reaction highly acid.

Lactose. Turbidity in bowl extending up into the closed arm. No precipitate but usually a thick scum is formed. Reaction highly acid.

Thermal death point. Organisms have survived 1 hour in the Arnold sterilizer and autoclaving to 15 pounds pressure. Killed by 16 pounds pressure.

Bacillus subtilis (Ehrenberg) Cohn

Synonyms. Vibrio subtilis Ehrenberg 1838; *Bacillus subtilis* Cohn 1872; *Bacillus subtilis* (Ehrenberg) Cohn, Migula 1900.

Considerable difference of opinion exists as to the correct interpretation of the somewhat puzzling literature concerning this organism. In this paper we have followed the views of Chester who has identified a number of organisms isolated in this country as the real *Bacillus subtilis* of Cohn, and who sent one of his isolations to our laboratory several years ago. It is one of the commonest organisms in milk, soil, dust and water. In morphology it is one of the smallest of the aerobic spore-bearing bacteria and is thus easily distinguished from *Bacillus cereus* with which it is most often confused.

Morphology. Small, thin, homogeneous bacilli measuring 0.375 by 1.5 to 2.5 microns in 24 hour agar cultures. Some-

what thicker and longer on glucose agar measuring 0.5 by 1.5 to 4 microns. Does not usually form threads on this medium. (Figures 4 and 5.)

Motility. Sluggishly motile in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early appearing within 24 hours on plain and glucose agar. They arise in the center or towards one end of the rods and are slightly greater in diameter than the rods, thus causing a distinct bulging. The free spores may retain bits of protoplasm at each end, often unequal in amount, giving the spore a characteristic appearance. Such spores measure about 0.5 by 0.875 microns. The spores rapidly lose their protoplasm, become more oval and measure about 0.5 by 0.75 microns.

Agar slant. Weakly refractive, glassy, membranous growth along line of inoculation, later spreading out over entire surface of agar. The surface is usually dry and hard, but in old cultures it becomes soft and smeary, but is always firmly attached to the agar from which it cannot be scraped off.

Agar stab. Little growth along the line of inoculation but a spreading, dry, membranous growth on the surface of the agar, extending to the wall of the tube.

Agar colonies. Surface colonies weakly refractive, spreading concentrically or in amoeboid fashion from small dense nuclei. Under the low power edges may be complete or finely crenate. If water of condensation be present one or two colonies frequently overgrow the entire plate. Under the low power the colonies are homogeneous and granular or irregular and gyrose. The deep colonies are punctiform and under the lower power lichen-like with irregular margins myceleoid in character. The colonies are usually membranous dry, hard, and glassy, and can be separated from the agar only with great difficulty.

Glucose litmus agar slant. Highly refractive growth verrucose or vesicular, with milky liquid in vesicles, not spreading. Parts of growth show distinct red pigment. Acid is produced in 24 hours, but is replaced by alkali in about ten days, medium turning deep blue.

Litmus glucose agar colonies. Irregular, spreading, bizarre surface colonies, usually more luxuriant than plain agar colonies. Under low power, irregular with entire edges or fuzzy, with myceleoid outgrowths from dense central nuclei. Deep colonies slightly irregular or punctiform. Under low power irregular myceleoid with filamentous edges. Medium first acidified then made alkaline.

Gelatin stab. Slow growth along line of inoculation and rather slow cup-shaped, surface liquefaction with scum production.

Gelatin colonies. Surface colonies round, homogeneous, spreading, thin and granular. Deep colonies yellowish brown, highly refractive. Under low power granular. Colonies may also show dense central nuclei and thin myceleoid filamentous growth extending in every direction through the medium. Gelatin liquefied.

Broth. Single isolated pellicles appear on the surface in 24 hours. In 48 hours these unite to form a thin branching scum, which gradually becomes more dense and tough. Medium grows turbid in first 24 hours, but later clears. Scum is precipitated as a whole in about ten days. This manner of scum formation is characteristic of *Bacillus subtilis.*

Peptone. Turbidity in the first 24 hours and gradual clearing with a flocculent precipitate. Scum on the surface formed in the same manner as on broth, but not so dense or tough. The pellicles often show chains and branching figures. Frequently the scum has a delicate pink color after about five days' growth.

Potato. Growth on potato characteristic. It is luxuriant and warty, having the appearance of many large and small dew drops scattered along the line of inoculation. In 48 hours a pink pigment collects on top of this growth and persists. In older cultures a decided rose-red line in the substance of the potato marks the limit of the growth. In ten days the vesicles dry down and only a reddish-brown dry growth remains on the discolored medium. Later the growth is moist and sticky.

Litmus milk. No change in 24 hours and sometimes none in 48 hours except that the milk becomes more alkaline. In three days the medium begins to clear from the surface, the deeper

parts remaining unchanged. Clearing progresses slowly, the supernatant fluid persisting as a grayish, pinkish or yellowish muddy medium. After a month at room temperature the medium may become very alkaline and turn deep blue-purple. Milk never coagulates.

Blood serum. Vesicular, dew-drop growth with pink color often very marked, in 24 hours. Vesicles dry down eventually leaving a hard wrinkled growth. Medium is not liquefied.

Fermentation tubes. Glucose. Turbidity in bowl and arm. Scum formation like that seen in broth. Highly acid.

Saccharose. Turbidity in bowl and arm with a fragile scum forming from pellicles in about two days. Acid production but not so marked as in glucose.

Lactose. Turbidity in bowl and extending up in the arm to the level of the medium in the bowl. Rest of the arm clear. Dense tough scum. Reaction alkaline.

Thermal death point. Spores survive steaming $1\frac{1}{4}$ hours in the Arnold sterilizer. Survive autoclaving up to and including 19 pounds pressure but usually destroyed by 20 pounds pressure.

Bacillus vulgatus (Flügge) Trevisan.

Synonomy. Bacillus mesentericus vulgatus Flügge 1886; *Bacillus vulgatus* Trevisan 1889; *Bacillus vulgatus* Eisenberg 1891; *Bacillus vulgatus* (Flügge) Migula 1900.

This organism was first described by Flügge in 1886 (Flügge, 1886) and is commonly known as the "potato bacillus." According to Chester it is identical with *Bacillus subtilis.* By the use of glucose agar and blood serum and by the careful observation of the cultural reactions, particularly in broth and on potato the species is easily separated from this organism. It is fairly common in Baltimore but by no means as frequent an isolation as are many of the other spore-bearers.

Morphology. Small homogeneous organisms usually distinctly larger than *Bacillus subtilis,* measuring 0.5 by 2 to 3 microns. Occasionally short forms 1.125 and long forms measuring 4 microns are seen on plain agar. On glucose agar the organisms

are thicker and much longer measuring often nearly 0.75 microns in thickness and 5 microns in length. (Figures 6 and 7.)

Motility. Active progressive and rotatory motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early appearing in 24 hours on plain and glucose agar. They arise in the center or towards one end of the rods but do not ordinarily bulge the rods appreciably. When free they are elongated and flattened and retain tags of protoplasm at each end. At times the protoplasm at one end is greater in amount then at the other. Such spores measure about 0.5 by 1.125 microns. As they lose their protoplasm they become cylindrical measuring about 0.5 by 1 micron. In general the spores are about the same width as the vegetative rods or only very slightly wider.

Agar slant. Moist profuse thick growth on agar, easily lifted or brushed from the surface of the medium with the platinum wire. Growth is usually white or cream white, spreads but little from the line of inoculation and is whitest at the edge where it is heaped up. When water of condensation washes over the agar many small, round colonies develop apart from the main growth. In some strains the agar growth is dry and fine wrinkles develop but the growth can always be lifted from the agar w'th a platinum loop.

Agar stab. Little growth along line of inoculation but rather dry wrinkled rooty growth spreads over the surface of the agar.

Agar colonies. Surface colonies round, waxy, highly refractive or spreading and amoeboid with greatest refraction at the edge of the advancing growth, where colonies are thickest. Under low power of the microscope edges entire. Deep colonies punctiform, round or elliptical. Under low power they are irregular, brown, slightly granular with entire or fuzzy edges.

Litmus glucose agar slant. Characteristic appearance. Luxuriant dry brown and abundantly wrinkled growth develops within 24 to 48 hours. The medium is acidified. After a few days the growth usually becomes moist and the wrinkles are obliterated while the medium becomes alkaline and turns deep blue.

Litmus glucose agar colonies. Superficial colonies are thick, highly refractive, waxy, with entire edges or spreading with irregular edges. They soon become dry and wrinkled in the center. Under low power opaque with entire edges. Deep colonies are punctiform, round, oval or irregular with crenated margins. Under low power opaque with irregular margins. Medium acidified at first then turned alkaline.

Gelatin. Stab gives cup-shaped and surface liquefaction with heavy scum production.

Gelatin colonies. Colonies round with highly refractive centers occasionally showing beautiful concentric rings. Under the low power the colonies have a granular appearance. Medium liquefied.

Broth. Turbidity within 24 hours and scum formation usually on second day. Medium gradually clears.

Peptone. Turbidity within 24 hours. Thin fragile scum after the lapse of several days. Medium gradually clears.

Potato. Characteristic appearance. Thick, white, gray or pink folds or wrinkles are formed within 24 to 48 hours often covering entire cut surface of potato. Later these folds dry down to a brown, reticulate mass. Potato usually discolored.

Litmus milk. Slight clearing of the milk just beneath the cream layer usually appears in 24 hours. This reaction rapidly intensifies with the production of a clear fluid colored deep Chinese-blue or purple. No acid production or coagulation. As the milk gets older complete peptonization occurs with the formation of a clear amber-colored fluid.

Blood serum. Thin dry abundant growth usually smooth, but sometimes wrinkled and pink. Growth later becomes moist and gives a suggestion of liquefaction.

Fermentation tubes. Glucose. Luxuriant growth in bulb gradually extending into the closed arm. An abundant scum is formed which may be quite wrinkled. Reaction acid.

Saccharose. Growth luxuriant in bowl but scanty in arm. Very thin scum may be formed after several days, but this may be lacking. Reaction varies from slight to marked acidity.

Lactose. Abundant growth in bowl with late scum production. No growth in closed arm. Reaction alkaline

Thermal death point. Organisms survived heating in broth in the Arnold sterilizer for one hour. Survived autoclaving up to and including 19 pounds pressure, but were destroyed by 20 pounds pressure.

Bacillus mesentericus (Flügge) Migula 1900

This organism was first described by Flügge in 1886 (Flügge, 1886) as a species distinct from *Bacillus mesentericus-vulgatus* and named *Bacillus mesentericus-fuscus*. We have isolated a number of organisms which correspond to the description given by Flügge and also by Chester. It is one of the less common of the aerobic spore-bearing bacteria but occurs in milk, soil, dust and water.

Morphology. Organisms about the same in morphology as *Bacillus mesentericus-vulgatus*. On agar cultures in 24 hours they are homogeneous rods measuring 0.5 by 1.5 to 3 microns. Sometimes shorter forms predominate in the cultures, a little over a micron in length. On glucose agar they are thicker and longer measuring 0.75 by 2 to 5 microns, with many long forms measuring 6 to 8 microns in length. (Figures 8 and 9.)

Motility. Active motility, progressive and rotatory, in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form in 24 hours on plain and on glucose agar. By the end of 48 hours they are very abundant. They appear in the center or towards one end of the rods and do not bulge the organism appreciably. The free spores are cylindrical and may retain equal bits of protoplasm at each end or this protoplasm may be unequal in amount giving a characteristic appearance to the spore. They measure about 0.5 by 1.125 microns. They rapidly lose their protoplasm and become slightly more oval, measuring 0.5 by 0.75 microns.

Agar slant. Soft white or cream-white growth somewhat translucent when old, spreading but little from the line of inoculation except in the presence of water of condensation. Easily lifted from the agar. Edges of growth irregular or serrate. Growth does not become dry or wrinkled.

Agar stab. Little growth along line of puncture, luxuriant growth on surface.

Agar Colonies. Superficial colonies round highly refractive with entire edges, or spreading and amoeboid. Under low power opaque with crenated edges. Deep colonies round and regular. Under low power slightly granular with crenated margins.

Litmus glucose agar slant. Thick, abundant, white or cream white to yellow growth spreading along the line of inoculation. Medium first turns acid but as growth becomes older it again becomes deep blue.

Litmus glucose agar colonies. Superficial colonies round, highly refractive, with entire edges or spreading and amoeboid with densest part of the growth along the advancing edge. Under low power of the microscope edges crenated. Deep colonies round or oval and under low power slightly granular with crenated margins. Medium first acidified and then made alkaline.

Gelatin stab. Cup-shaped or surface liquefaction and scum production.

Gelatin colonies. Colonies dense with liquefaction centers and granular ring at the edges of a cup-shaped liquefaction.

Broth. Turbidity and a rather fragile scum appears late. Medium then clears.

Peptone. Turbidity with small patches of surface growth. Medium soon clears.

Potato. Growth abundant, moist, brown with finely wrinkled or lichen-like appearance in the majority of instances. At times the fine wrinkling is lacking and only a thick, moist, brown, mealy growth is produced.

Litmus milk. Slow peptonization with the production of a lilac color turning to amber. In a few weeks digestion is complete and only a white sediment is left behind. No acidification No coagulation.

Blood serum. Thin, white, dry, at times finely wrinkled growth which later becomes yellowish and moist. Suggestion of liquefaction, but this is never complete.

Fermentation tubes. Glucose. Turbidity and scum in bulb and turbidity in closed arm. Reaction acid.

Saccharose. Turbidity in open bulb and usually no scum. Turbidity in closed arm. Reaction acid.

Lactose. Turbidity in open bulb. No scum. Arm clear. Reaction alkaline.

Thermal death point. Spores survive one hour's heating in Arnold sterilizer and autoclaving at 19 pounds pressure. Killed by 20 pounds pressure.

Bacillus globigii Migula.

This organism was originally described by Globig (1888) as *Bacillus mesentericus-ruber.* A culture was obtained from Kral's Laboratory in Vienna which has the same cultural reactions as those given by Globig.

Morphology. Homogeneous bacilli measuring 0.5 by 2 to 3 microns in 24 hours agar cultures. On glucose agar the organisms are longer and slightly thicker often growing out into long chains but short forms are also frequently seen (Figure 14).

Spore formation. Spores are formed very sparsely and at a late period in the present culture. They are usually seen only in 16 to 18 days growth and are then characteristic mesentericus spores.

Motility. Actively motile in 24 hour cultures.

Staining properties. Gram-negative.

Agar slant. Thin, spreading, glassy, soft, yellowish-white growth along line of inoculation.

Agar stab. Slight uniform growth along line of puncture with spreading amoeboid surface growth.

Agar colonies. Dense, soft, white amoeboid colonies similar to those of *Bacillus mesentericus.*

Litmus glucose agar slant. Thick, narrow, white growth along line of inoculation. The medium shows an acid reaction.

Litmus glucose agar colonies. Thick, round, raised, soft colonies later turning yellowish and rarely pinkish. Medium acidified.

Gelatin stab. Growth along line of inoculation and slight surface growth with liquefaction of the gelatin.

Gelatin colonies. Surface colonies round, granular, punctiform with slow liquefaction. Some of the larger colonies are spreading and have a glassy surface. Deep colonies punctiform, spherical with dense centers.

Broth. Slight turbidity with no surface growth but a flocculent precipitate. Medium is eventually turned dark yellow.

Peptone. Slight turbidity with no surface growth and no precipitate.

Potato. Yellow, moist growth becoming a reddish brown. Medium is discolored.

Milk. No change in 48 hours. In twenty days the medium shows an acid reaction with the precipitation of a white sediment.

Blood serum. Thick, transparent spreading growth with irregular edges.

Fermentation tubes. Glucose. Turbidity in open bulb. No scum. No growth in closed arm. Reaction acid.

Saccharose. Turbidity. No scum. No growth in closed arm. Reaction alkaline.

Lactose. Turbidity. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. Spores withstood one hour's sterilizing in the Arnold sterilizer, survived autoclaving at 15 pounds pressure but were killed by 16 pounds pressure.

Bacillus aterrimus Lehmann & Neumann.

This organism was originally described by Biel (1896) and named by Lunt (1896) *Bacillus mesentericus-niger.* It is not uncommon in milk, soil, and the intestinal contents of man.

Morphology. Bacilli similar to *Bacillus vulgatus* in morphology. On plain agar they are homogeneous with blunt ends and measure about 0.5 by 2 to 3 microns in dimensions. On glucose agar they are thicker and longer measuring 0.75 by 2 to 4 microns but at the same time shorter forms are frequent measuring 0.75 by 1.5 microns. (Figures 10 and 11.)

Spore formation. Spores are formed early appearing in 24 hours on plain agar. They form in the center or towards one

end of the rods which do not swell appreciably. When free they may retain spurs of protoplasm at each end unequal in quantity and measure about 0.5 by 1.5 microns. The spores rapidly lose their rims of protoplasm and are then oval to cylindrical measuring 0.5 by 0.75 microns.

Motility. Actively motile in 24 hour cultures.

Staining properties. Gram-positive.

Cultural reactions. This organism is identical with *Bacillus vulgatus* in all its cultural reactions except that it imparts a distinct color to the various media. This color varies from a steel grey to a brown or black and is best seen on solid media. It is very pronounced on potato where the characteristic folds of the "*vulgatus*" are converted to thick black wrinkling bands.

Thermal death point. The spores resist an hour's steaming in the Arnold sterilizer and 15 pounds pressure in the autoclave. They are destroyed by 16 pounds pressure.

Bacillus niger Migula 1900

This organism was first described by Gorini (1894) in 1894 as *Bacillus lactis-niger* and is closely related to the preceding organism. A culture obtained from Kral's Laboratory shows the following reactions.

Morphology. Bacilli with homogeneous protoplasm and blunt or rounded ends measuring 0.375 to 0.75 by 1.5 to 3 microns in 24 hour agar cultures. No change in morphology on glucose agar. (Figures 12 and 13.)

Spore formation. Spores are formed in 24 hours on plain agar and in 48 hours on glucose agar. They appear in the center or towards one end of the rods and are oval or cylindrical in shape. The free spores may retain protoplasm at both ends and are typical of the "mesentericus" group. They measure 0.75 to 1 by 1.125 to 1.25 microns in dimensions.

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Cultural reactions. This species has the general cultural reactions of *Bacillus mesentericus.* It grows on agar as a rather

thick moist mass with a silvery sheen which shows black areas at the edges and in old cultures imparts a black tone to the agar. It liquefies gelatin rapidly, produces a faint acidity in milk which it first coagulates and then slowly digests. On glucose agar it tends to wrinkle slightly. It produces a faint acid in glucose, saccharose and lactose fermentation tubes. On potato it grows as a raised brown mass and it also produces a brownish growth on blood serum.

Thermal death point. The spores withstand boiling one hour in the Arnold sterilizer and a pressure of 20 pounds in the autoclave. They are destroyed by a pressure of 22 pounds.

Bacillus pumilus Gottheil 1901

An organism described by Gottheil (1901) in 1901 as *Bacillus pumilus* is regarded by Chester as identical with *Bacillus mesentericus.* A culture of *Bacillus pumilus* received from Kral's collection in Vienna has all the morphological, tinctorial, developmental and cultural reactions of this species.

Bacillus mycoides Flügge 1886

This organism was first described by Flügge (1886) in 1886 and has since then been given other names by various authors. It is not the same as *Bacillus ramosus-liquefaciens* of Prausnitz which is a distinct species. *Bacillus mycoides* is quite common in Baltimore and is present in milk, water, soil, and dust.

Morphology. In young cultures 6 to 8 hours old on plain agar the organisms are homogeneous with square ends and measure usually a little more than 0.5 micron in width by 3 to 6 microns in length. They are distinctly thinner and longer than *Bacillus cereus.* As the organisms mature the protoplasm appears more granular and a characteristic arrangement in short and long chains is seen. They then resemble the anthrax bacillus. On glucose agar the bacilli are thicker, 0.75 to 1 micron, and usually about the same length. On this medium the protoplasm is converted into globular bodies which do not take the stain and which are similar to those seen in *Bacillus cereus.* In certain

instances the organisms seem to be made up of a network of fine strands in which the globular bodies hang suspended. Often the chains are curled or curved upon themselves. Old cultures show an abundance of swollen involution forms, which seem to have a skein-like arrangement. (Figures 22, 23 and 24.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form early, appearing first as small refractile bodies in the centers or towards one end of the organisms usually at the end of 24 to 48 hours. Gradually the organisms swell and the spores at the same time increase in size and at this stage a long chain of organisms each containing a spore may often be seen. The protoplasm soon disintegrates leaving a rim about the spore which is round or oval or slightly rectangular. Such spores measure 0.75 to 1 by 1.125 microns. Other spores are more definitely elongated and may measure 0.75 to 1 by 1 to 2 microns. The spores often remain attached to each other in short or long chains. The spores vary more in size than do others of this group and may show small forms 0.375 to 0.5 by 0.5 to 1 and large forms measuring 1.125 by 2 lying side by side.

Slant agar. Filamentous rhizoid growth spreading from the line of inoculation and extending into the agar. This growth is at first glassy and glistening, but later grows dull and soft. Appearance on agar characteristic.

Agar stab. Faint arborescent growth along line of inoculation with a surface development in concentric zones.

Agar colonies. Surface colonies spread from dark dense nuclei and show dense, rhizoid peripheries extending into the agar on all sides. Under low power the periphery of the colony is found to be composed of parallel strands of growth. Deep colonies have almost the same appearance and always exhibit the spreading peripheral myceleoid outgrowths.

Litmus glucose agar. Thin membranous myceleoid growth later becoming branched and reticulate. Growth at first moist and white, later becoming pale yellow. Medium first acidified and then turned deep blue.

Litmus glucose agar colonies. Surface colonies thin, round or irregular. Under low power found to consist of masses of matted filaments with usually dense central nuclei, from which single or parallel strands extend into the agar in every direction for long distances. Deep colonies exhibit the same small, punctiform and matted myceleoid growth, under lower power. Medium first acidified and then made alkaline.

Gelatin stab. Filamentous growth along line of inoculation with surface liquefaction.

Gelatin colonies. Colonies consist of dense central nuclei with matted edges from which long strands emerge. The colonies present a peculiar appearance like a chestnut burr.

Broth. No turbidity but a firm scum forms which is soon precipitated.

Peptone. No turbidity, but a flocculent suspension and a firm scum which is soon precipitated.

Potato. Mealy white, later becoming brownish.

Litmus milk. Slow peptonization to an amber-colored fluid. No acidification. No coagulation.

Blood serum. Dry, myceleoid interlacing luxuriant growth. No liquefaction.

Fermentation tubes. Glucose. Flocculent growth in bowl and arm. Scum forms and is soon precipitated. Reaction acid.

Saccharose. Flocculent in bowl and in arm. Scum is formed and precipitated. Some cultures produce moderate acidity. Others produce no acid.

Lactose. Growth in open bulb with a slight extension into arm. Scum formed and soon precipitated. Reaction alkaline.

Thermal death point. Spores survived one hour in the Arnold sterilizer and 15 pounds pressure in the autoclave. Destroyed by 16 pounds pressure.

Bacillus megatherium De Bary

This organism was originally found and named by De Bary (1884, 1887) and has since beeen described under a variety of names by a number of authors. It is one of the most common of

the spore-bearing bacteria and has been found in dust, soil, milk, water, and as a laboratory contamination. The present description applies to cultures obtained from the Kral collection, and from the American Museum, and to over a hundred of our own isolations.

Morphology. These are the largest of the spore-bearing organisms. On plain agar in young cultures, from 8 to 24 hours old, they are long and thick with homogeneous or slightly granular protoplasm, measuring 0.75 to 1.25 by 3 to 9 microns. On glucose agar they are even thicker measuring 1.25 to 1.5 in width. On both media long forms occur but especially on glucose agar. These may measure 30 to 45 microns in length and may show homogeneous protoplasm without evident segmentation. The protoplasm of the organism is at first homogeneous, but by the end of 24 to 48 hours it is converted into a mass of globular bodies resistant to the stains. These globular bodies are clear, highly refractile, bulge the organism somewhat, and are quite numerous six to eight appearing in each rod. They thus give the organism a peculiar and characteristic appearance. They show most markedly on glucose agar but are also present on plain agar where they can best be demonstrated by decolorizing an over-stained preparation. Their nature is not clear as they do not take any special bacterial stains. Shadow or transparent forms appear in *Bacillus megatherium* early, both on plain and glucose agar. These measure 1.125 to 1.5 by 4 to 10 microns, take the stain very faintly and show peculiar bodies of agglomerated protoplasm at the sides or sometimes at the ends. These transparent forms are often thicker and longer and may even measure 2 by 40 to 45 microns. Occasionally they are distinctly oval with rounded ends measuring about 1.5 by 4 microns and show a small bunch of cytoplasm at the side. When these forms are in chains they are exactly like the original pictures of De Bary. (Figures 31, 32, 33, 34, 35, 36, 37).

Motility. Active progressive and rotatory motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed abundantly on plain

agar in 24 hours and on glucose agar in 48 hours. They appear in the center or slightly towards one end of the rods and are usually of the same diameter but may be slightly thicker. Sometimes two spores seem to arise in one rod but these may possibly be in a rod just prior to division. In general each rod has a single spore. The spores occasionally lie obliquely in the rods. Frequently two spores are at opposite ends of rods lying in juxtaposition and these may remain attached in chains and present a characteristic appearance. The free spores retain protoplasm at the ends for some time. When this is unequal in amount the spore has somewhat the shape of a tennis racket and handle. The free spores are oval to cylindrical and measure 0.75 to 1.125 by 1.5 to 2 microns. They are often flattened on one side having an appearance described as kidney shaped or reniform. The spores show great variations in size more so than do those of the other members of this group.

Slant agar. Thick, raised, soft, white or cream-colored growth which shows a pink tinge by reflected light, with many small, minute pellucid areas. As the cultures get older the growth becomes pale yellow.

Agar stab. Slight growth along line of inoculation, heaped up and spreading slightly on surface. Later surface growth becomes slightly pinkish.

Agar colonies. Surface colonies round, thick, white or cream-colored, highly refractive, turning pale yellow or yellowish-brown in old cultures. Under low power slightly granular, brownish yellow, with entire margins. Deep colonies punctiform. Under low power round or irregular with entire edges, brown and granular.

Litmus glucose agar slant. Thick, luxuriant growth along line of inoculation, at first white and then pale yellow or cream-colored. Medium is first acidified but later becomes alkaline and changes from a dark blue to a smoky brown while the growth becomes a dark gray or smoky brown.

Litmus glucose agar colonies. Large, round, raised surface colonies, cream colored to pale yellow, with heaped up central nuclei. Under low power dark, slightly granular with entire

edges. Deep colonies punctiform. Under low power dark, irregular, bizarre, with entire edges. Medium is acidified and then made alkaline.

Gelatin stab. Funnel-shaped liquefaction. No scum.

Gelatin colonies. Round colonies with concentric zones of growth. Under low power cloudy central nuclei with filamentous peripheries.

Broth. Turbidity but no scum.

Peptone. Turbidity but no scum.

Potato. Thick, white, mealy growth later becoming pale or cream yellow.

Litmus milk. No change within 24 hours then a gradual peptonization with the production of a port-wine colored fluid. No acidification. No coagulation.

Blood serum. Thick, white, moist, heavy growth cream white to yellow in color. No liquefaction.

Fermentation tubes. Glucose. Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

Saccharose. Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

Lactose. Slight turbidity in open bulb. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. Spores withstood 1 hour steaming in the Arnold sterilizer and 18 pounds pressure in the autoclave. Killed by 19 pounds pressure.

Bacillus petasites Gottheil 1901

This organism was described originally by Gottheil (1901) in 1901. Its chief point of differentiation from *Bacillus megatherium* is that it produces a distinct yellow pigment on artificial media, particularly on plain agar and on potato. It is extremely common, having been found in dust, soil, water, milk, and various milk products. The present description applies to a culture from the Kral collection and to over a hundred of our own isolations.

Morphology. The organisms do not differ appreciably in morphology from *Bacillus megatherium.* They are homogeneous

or slightly granular rods measuring 0.75 to 1.5 by 3 to 6 microns on plain agar in young cultures (8 to 24 hours), and 1 to 1.75 by 3 to 6 on glucose agar. Long forms measuring 12 to 25 microns are seen on plain and on glucose agar. Shadow forms with faintly staining protoplasm, like those seen in *Bacillus megatherium* are common as well as the peculiar refractile globular bodies. (Figures 38, 39, 40, 41 and 42.)

Motility. Active progressive and rotatory motility in young cultures.

Staining reactions. Gram-positive.

Spore formation. Spores are formed abundantly in 24 hours on plain and on glucose agar. The spores are oval to rectangular, of about the same width as the rods from which they spring and frequently form long chains. The free spores may retain tags of protoplasm but soon lose them and then show great variations in size and shape. They may be nearly round, oval, rectangular and reniform and measure 0.75 to 1 by 1.5 to 2 microns.

Agar slant. Thick, moist, abundant mealy growth at first slightly pinkish by reflected light, then becoming bright lemon yellow. Agar slightly discolored.

Agar stab. Slight growth along line of inoculation with heaped up yellowish growth on surface.

Agar colonies. Surface colonies thick, white or yellow, highly refractive. Under low power dark, granular with entire or myceleoid edges. Deep colonies punctiform. Under low power irregular, with irregular edges showing myceleoid, rooty or fuzzy edges.

Litmus glucose agar. Luxuriant, thick, heaped-up growth at first yellow then assuming an orange and then a dark-brown color. Reaction of medium first acid then alkaline. It eventually becomes smoky-brown.

Litmus glucose agar colonies. Surface colonies round, regular and thick or thin and spreading. Under low power granular with entire edges. Deep colonies punctiform. Under low power granular, irregular, with fuzzy edges. Reaction of medium acid at first then alkaline.

Gelatin stab. Growth along line of inoculation with funnel-shaped surface liquefaction. No scum formation.

Broth. Turbidity. No scum. Medium eventually becomes yellow.

Peptone. Turbidity. No scum.

Potato. Thick, mealy, bright yellow growth gradually becoming dark yellow.

Litmus milk. No change in 24 hours then a gradual peptonization with the production of a port-wine-colored fluid.

Blood serum. Thick, dry, yellowish, moist growth becoming pale to bright yellow. No liquefaction.

Fermentation tubes. Glucose. Slight turbidity in bulb. No scum. No growth in closed arm. Feeble acid production.

Saccharose. Slight turbidity in bulb. No scum. No growth in closed arm. Faint acid production.

Lactose. Slight turbidity in bulb. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. Spores survived steaming in Arnold sterilizer 30 minutes, but were killed by 1 hour exposure. Withstood 19 pounds pressure in autoclave but were killed by 20 pounds pressure.

Bacillus tumescens Zopf 1885

This organism was described by Zopf in 1885 (Zopf, 1885). A culture received from the Kral collection and another received from the American Museum agree in their morphological, developmental, tinctorial and cultural features all of which are identical with those of *Bacillus megatherium.* (Figures 45, 46 and 47.)

Bacillus graveolens Gottheil 1901

This organism was described in 1901 by Gottheil (1901) as a new species. A culture from the Kral collection in Vienna has all the cultural reactions of *Bacillus megatherium.* Morphologically it is about the same size, forms spores in the same way, is Gram-positive, produces globular bodies on plain and glucose agar and undergoes involution with the formation of shadow or

washed-out forms. It shows however a distinct tendency to produce curved or spiral forms. On the basis of this one characteristic it is hardly justifiable to make it a distinct species. It should be noted that this use of the term "*graveolens*" is probably incorrect since a *Bacterium graveolens* was described by Bordoni Uffreduzzi (1886) in 1886. This was a small non-sporulating bacillus producing a green pigment. (Figures 43 and 44.)

Bacillus cohaerens Gottheil 1901.

This organism was described by Gottheil (1901) in 1901 but according to Chester it is identical with *Bacillus simplex*. The culture of *Bacillus cohaerens* received from the Kral collection is distinct from *Bacillus simplex* and is represented by four organisms isolated in Baltimore, one from milk and three from soil. The present description applies to all five strains.

Morphology. Small, rather uniform homogeneous organisms with rounded ends, measuring 0.375 to 0.5625 by 0.75 to 2.25 microns in 24 hour cultures on plain agar. On glucose agar the bacilli are thicker and longer measuring 0.5625 to 0.75 by 2 to 5 microns. On both media shadow forms appear early often in 24 hours. These are made up of faintly-staining protoplasm with deeply-staining particles in various positions, at the ends, towards the center, or at the periphery. (Figures 15, 16, and 17.)

Motility. Actively motile in 24 hour cultures.

Staining properties. Gram-positive.

Spore formation. Spores were formed slowly and sparsely in the Kral culture and in one of ours. They appeared in about 10 days, were oval or elliptical, arose in the centers of the rods which were slightly bulged on sporulation. The free spores were very delicate and stained with difficulty. They measured about 0.5625 by 0.75 microns. In a more recent isolation of our own the spores appeared in 48 hours, were central or excentric, bulged the rods and later retained distinct rims of protoplasm, measuring 0.75 by 1.5 to 1.5 microns. Later the spores lost their protoplasm, became more oval and measured 0.5 to 0.5625 by 0.9375 to 1.25 microns. Rarely the spores retained unequal

bits of protoplasm at the ends and then they resembled the mesentericus spores slightly.

Agar slant. Thin, soft spreading, whitish growth later becoming yellow. Easily scraped off the agar.

Agar stab. Faint growth along line of inoculation and spreading on the surface, thick and whitish in old cultures.

Agar colonies. Surface colonies round or bizarre, thick, white. Under low power granular with dense central nuclei. Edges entire. Deep colonies punctiform. Under low power irrregular, with entire edges.

Litmus glucose agar. Thick, soft, whitish growth along line of inoculation becoming yellowish and irregularly heaped up. Medium quite markedly acidified.

Litmus glucose agar colonies. Surface colonies round or irregular, thick, whitish. Under low power granular and frequently show dense central nuclei with thin peripheries showing regular edges. Deep colonies punctiform. Under low power irregular with irregular edges. Reaction of medium acid.

Gelatin stab. Faint growth along line of inoculation with surface liquefaction and scum production.

Gelatin colonies. Thin, circular colonies, under low power granular.

Broth. Turbidity at first, then the medium clears and a dense surface growth appears which shows many clear, globular masses like globules of fat floating on the surface.

Peptone. Turbidity with a faint fragile scum.

Potato. Thin, spreading, moist, yellow growth.

Litmus milk. Slow decolorization of the litmus with peptonization and the production of an amber-colored fluid.

Blood serum. Thin, whitish growth. No liquefaction. May appear finely wrinkled.

Fermentation tubes. Glucose. Turbidity in bowl with surface growth and flocculent precipitate. Arm clear. Reaction acid at the end of 2 to 3 days.

Saccharose. Turbidity in bowl with slight surface growth. Arm clear. Acidity at the end of 2 to 3 days.

Lactose. Turbidity in bowl with very slight surface growth. Arm clear. Reaction alkaline.

Thermal death point. In one isolation the spores survived one hour steaming in the Arnold sterilizer; and withstood 18 pounds pressure in the autoclave but were killed by 19 pounds pressure. In another isolation from soil the spores survived 14 pounds pressure in the autoclave but were killed by 16 pounds. They survived one hour steaming in the Arnold.

Bacillus simplex Gottheil 1901

This organism was described by Gottheil (1901) in 1901 as a distinct species. According to Chester it is the same as *Bacillus cohaerens* of Gottheil. Cultures of both organisms have been received from Kral's Laboratory in Vienna and can easily be differentiated. The present description applies to the Kral culture and to an organism obtained from soil by Dr. Laubach. The species is evidently one of the rare spore-bearing organisms.

Morphology. In the Kral culture the organisms are large homogeneous rods with rounded ends, measuring usually 0.5625 to 0.75 by 3 to 4.5 microns. At times much thicker forms are seen approximating 1.125 micron in thickness while longer forms 6 microns in length are not uncommon. The organisms often grow out into long threads or filaments 10 to 12 microns in length, especially on glucose agar. Even in young cultures the homogeneous rods lose their protoplasm and are converted into peculiar shadow forms. These are made up of a very faintly staining protoplasm in which denser aggregations of cytoplasm appear. Such forms measure 1.125 to 1.25 by 12 to 15 microns in dimensions. On glucose agar the organisms have the same morphology but may show an abundance of shadow forms. Involution and shadow forms are very abundant in old cultures. In our own isolation the organisms, while somewhat smaller, did not differ appreciably in morphology, measuring 0.5 to 0.5625 by 1.5 to 2.5 microns but also showing both the thicker and longer forms seen in the Kral culture and the characteristic shadow and involution forms. Long forms were also very common on glucose agar. (Figures 18, 19, 20, and 21.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore formation. In the Kral culture the spores were at first formed very slowly appearing only after the lapse of 15 to 16 days. Subsequently after repeated transfers, spore formation became more active and spores were often formed in 24 hours. They appeared in the centers or towards one end of the rods, were no thicker than the rods from which they sprung, and were cylindrical or almost rectangular in shape. They retained rather thick walls of protoplasm for some time and measured 0.5625 by 1.125 to 1.25 microns. In our own isolation the spores were formed in 48 to 72 hours in the same way as in the Kral culture but were a trifle smaller measuring 1.375 to 0.5 by 0.75 to 1 micron.

Agar slant. Thin, translucent, slightly yellowish gelatinous growth, gradually becoming denser and developing occasionally a dry slightly wrinkled surface. Single accessory colonies not uncommon at the edges of the main growth.

Agar stab. Slight uniform growth along line of puncture with a thick circular surface growth.

Agar colonies. Surface colonies thin, translucent, amoeboid developing from pin-point centers. Under low power granular. Deep colonies round or oval, regular, granular, with clean or rarely irregularly fuzzy edges.

Litmus glucose agar. Thick, abundant yellowish-white, heaped up growth with serrated margins. Medium faintly acidified in old cultures.

Litmus glucose agar colonies. Superficial colonies thin, smooth, white and soft. Under low power granular, edges irregular but entire. Deep colonies punctiform. Under low power irregular with irregular rarely fuzzy margins. A trace of acid usually produced.

Gelatin stab. Faint growth along line of inoculation with cup-shaped surface liquefaction.

Gelatin colonies. Round, thick, whitish colonies with concentric rings and sharply defined edges. Medium liquefied.

Broth Faint turbidity, slight sediment, no scum but rarely a faint ring growth along side of tube.

Litmus milk. Gradual clearing with production of straw-colored fluid in the Kral culture. In our own isolation a gradual

clearing to a port-wine fluid. No coagulation. Later straw-colored.

Peptone. Faint turbidity and sediment with rarely a slight ring growth.

Potato. Thick, moist, abundant, gelatinous, yellowish-brown growth.

Blood serum. Thin, spreading, whitish growth. No liquefaction.

Fermentation tubes. Glucose. Turbidity in open bulb. No scum, arm clear. Reaction neutral or slightly acid.

Saccharose. Faint turbidity in bulb. No scum. Arm clear. Reaction alkaline.

Lactose. Faint turbidity in bowl. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. In the Kral culture the spore survived steaming in the Arnold sterilizer for 15 minutes. They withstood a pressure of 15 pounds in the autoclave but were destroyed by 16 pounds pressure. In our own isolation the spores survived 10 pounds in the autoclave but were killed by 12 pounds pressure. They survived 15 minutes steaming in the Arnold sterilizer but were killed by 30 minutes steaming.

Bacillus fusiformis[2] Gottheil 1901

This organism was first described by Gottheil (1901) in 1901. A transfer from Gottheil's original was obtained from Kral's Laboratory in Vienna. Fourteen organisms corresponding closely to Gottheil's isolation were obtained in Baltimore, two from milk, four from dust, two from water, five from soil and one from contaminated hirudin. The present description applies to all of them.

[2] *Bacillus fusiformis* has practically the same morphology and the same cultural reactions as the organism described in 1909 by Jordan and Harris as the cause of milksickness and named by them *Bacillus lactimorbi* (Journal of Infectious Diseases, Vol. 6, No. 4, September 20, 1909, p. 401). A culture of *Bacillus lactimorbi* received from the Winslow collection in New York does not differ appreciably in its reactions from the strains of *Bacillus fusiformis* in our laboratory. Without a thorough study of pathogenicity, however, it is impossible to state whether the organisms found by us are identical with *Bacillus lactimorbi* or not.

Morphology. Thick stubbed homogeneous organisms with round or pointed ends usually appearing as single cells or in twos. No chain formation. On 24 hour plain agar cultures they measure 0.5 to 0.75 by 1.5 to 2 microns. Organisms not increased in size on glucose agar and protoplasm remains homogeneous. Sometimes long forms 6 to 8 microns appear in old cultures. (Figures 48, 49, 50 and 51.)

Motility. Active progressive and rotatory motility in 24 agar cultures.

Staining properties. Gram-negative.

Spore formation. Spores form early appearing in 24 hours on both plain and glucose agar. They are round, greater in diameter than the organisms from which they spring, and are usually located at the ends of the rods in a terminal or subterminal position. They thus give a clavate or club-shaped appearance to the rods which resemble somewhat the tetanus bacillus. The spores may also be central and the rods thus become fusiform in shape. The free spores may retain spurs of protoplasm assuming a peculiar diamond shape or may appear naked. They vary in diameter from 0.5 to 1 micron and are occasionally swollen equaling 1.5 microns in thickness.

Agar slant. Thick white rather dry growth in 24 hours, becoming distinctly yellow or cream-colored in old cultures. Easily scraped from medium.

Agar stab. Faint line growth and non-spreading surface growth.

Agar colonies. Superficial colonies may be round, regular thick and opaque, or thin and spreading. Under low power they show dark central nuclei and thinner margins with clean-cut edges. Older cultures thick and heaped up. Deep colonies small and fine, under low power dark, opaque, round or irregular.

Glucose agar. Thick dry growth with heaped-up edges becoming thicker and granular in old cultures. Reaction alkaline.

Glucose agar colonies. Superficial colonies thick, irregular spreading and heaped up. Under low power granular with irregular fuzzy margins. Deep colonies opaque under low power showing irregular fuzzy edges. Older colonies thicker and more bizarre-shaped. Reaction alkaline.

Gelatin stab. Growth along line of inoculation with cup-shaped or funnel-shaped liquefaction. Dense turbidity in the liquefied gelatin with a thick scum. Gelatin may be faint pink in color.

Gelatin colonies. Small fine colonies round and regular or irregular and spreading. Under low power they show fine hairy outgrowths. Gelatin slowly liquefied.

Broth. Turbidity and fine sediment. No scum.

Peptone. Turbidity and fine sediment. No scum.

Potato. Faint yellow growth becoming yellowish brown in old cultures.

Litmus milk. Gradual reduction of the litmus and slow but complete digestion of the proteins. No coagulation.

Blood serum. Non-spreading cream yellow growth becoming yellowish brown in old cultures. No liquefaction.

Fermentation tubes. Glucose. Turbidity in bowl. Arm clear. No scum. Reaction alkaline.

Saccharose. Reactions the same.

Lactose. Reactions the same.

Thermal death point. Spores destroyed by steaming 15 minutes in the Arnold sterilizer. They survive 7½ pounds in the autoclave but are destroyed by 10 pounds pressure.

Bacillus terminalis Migula 1900

This organism was first obtained by Flügge (1894) in 1894 and called by him, No. XII. It was subsequently correctly named *Bacillus terminalis* by Migula and still later named *Bacillus lacteus* by Chester (1901). On two occasions we have isolated organisms which have the same morphology and method of spore-formation as *Bacillus terminalis* but differ slightly in cultural reactions. It does not seem wise to make a new species since our strains may represent merely attenuated varieties of Flügge's organism. The following description is taken from our own isolations and the points of differentiation between them and Flügge's original isolation are indicated.

Morphology. Long thin bacilli with slightly granular protoplasm measuring 0.375 by 2.25 to 4 microns in 24 hour agar cul-

tures. On glucose agar the organisms retain the same diameter but grow out into long chains which often assume spiral arrangements. (Figure 52.)

Spore formation. Spores are formed slowly seldom appearing before 48 hours. They are cylindrical, thicker than the rods from which they spring, terminal or sub-terminal, giving the organisms a clavate or club-shaped appearance. Free spores are 0.75 by 1.5 microns in dimensions.

Motility. Active motility in 24 hour cultures.

Staining properties. Gram-negative.

Agar slant. Thin spreading smooth glistening growth with gradual darkening of the agar.

Agar stab. Faint growth along line of puncture and on the surface at the point of inoculation.

Agar colonies. Colonies grow slowly appearing only after 3 to 4 days. They are round, regular, under low power showing central nuclei with thin spreading peripheries. Deep colonies apt to be irregular under low power, showing clean-cut or entire edges.

Glucose agar. Faint white filmy growth with an alkaline reaction.

Glucose agar colonies. Thin slow-growing spreading surface colonies, under low power showing dense central nuclei and thin margins. Deep colonies punctiform, under low power slightly granular with irregular margins. Reaction alkaline.

Gelatin stab. Growth along line of inoculation and slow cup-shaped liquefaction.

Gelatin colonies. Colonies on the surface show dense central nuclei and concentric spreading peripheral margins. Deep colonies punctiform and tend to show same arrangement. Under low power edges entire.

Broth. Slight turbidity. No scum. No sediment. Fragile scum described by Flügge.

Peptone. Slight turbidity. No scum. No sediment.

Potato. No visible growth in our isolations. Faint moist growth gradually becoming thicker and yellowish, noted by Flügge.

Milk. No change produced by our strains. Slow peptonization described by Flügge.

Blood serum. Thin transparent spreading growth, pale yellow to yellowish-brown. No liquefaction. Slight sinking-in of the growth mentioned by Flügge.

Fermentation tubes. Glucose. Faint turbidity in bowl. No scum. No growth in closed arm. Reaction alkaline.

Saccharose. Appearance the same. Reaction alkaline.

Lactose. Appearance the same. Reaction alkaline.

Thermal death point. Spores survived 10 pounds in autoclave but were killed by 15 pounds pressure.

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ILLUSTRATIONS

The illustrations were drawn by Mrs. W. W. Ford and Mr. W. P. Didusch from preparations illustrating the different phases in the morphology of the various species. The preparations were always stained by gentian violet and drawn from a Zeiss microscope with a 1/12 oil immersion lens and a compensating ocular No. 8, giving a magnification of 1080 diameters. The attempt was made to show the morphology of the vegetative cells which comes out best in certain species at 6 to 7 hours and in others at 22 to 24 hours, the method of spore-formation which varies in the time in which it appears, and the changes which occur in the organism when grown on carbohydrate media.

EXPLANATION OF PLATES

PLATE 1

FIG. 1. *Bacillus coli.* Plain agar, 24 hours
FIG. 2. *Bacterium anthracis.* Plain agar, 24 hours

PLATE 2

FIG. 3. *Bacterium anthracis.* Plain agar, 4 days
FIG. 4. *Bacillus subtilis* from milk. Plain agar, 20 hours

PLATE 3

FIG. 5. *Bacillus subtilis* from milk. Glucose agar, 24 hours
FIG. 6. *Bacillus vulgatus* from milk (*Bacillus mesentericus vulgatus*). Plain agar, 20 hours

PLATE 4

FIG. 7. *Bacillus vulgatus* from milk (*Bacillus mesentericus vulgatus*). Glucose agar, 24 hours
FIG. 8. *Bacillus mesentericus* from soil (*Bacillus mesentericus fuscus*). Plain agar, 72 hours

PLATE 5

FIG. 9. *Bacillus mesentericus* from soil (*Bacillus mesentericus fuscus*). Glucose agar, 48 hours
FIG. 10. *Bacillus aterrimus* from human intestinal contents (*Bacillus mesentericus niger*). Plain agar, 20 hours

PLATE 6

FIG. 11. *Bacillus aterrimus* from human intestinal contents (*Bacillus mesentericus niger*). Glucose agar, 48 hours
FIG. 12. *Bacillus niger* from Kral (*Bacillus lactis niger*). Plain agar, 48 hours

PLATE 7

FIG. 13. *Bacillus niger* from Kral (*Bacillus lactis niger*). Glucose agar, 48 hours

FIG. 14. *Bacillus globigii* from Kral (*Bacillus mesentericus ruber*). Plain agar, 20 hours

PLATE 8

FIG. 15. *Bacillus cohaerens* from milk. Plain agar, 7 hours

FIG. 16. *Bacillus cohaerens* from soil. Plain agar, 6 hours

PLATE 9

FIG. 17. *Bacillus cohaerens* from soil. Plain agar, 24 hours

FIG. 18. *Bacillus simplex* from Kral. Plain agar, 5 hours

PLATE 10

FIG. 19. *Bacillus simplex* from Kral. Plain agar, 20 hours

FIG. 20. *Bacillus simplex* from soil. Plain agar, 24 hours

PLATE 11

FIG. 21. *Bacillus simplex* from soil. Plain agar, 3 days

FIG. 22. *Bacillus mycoides* from cow dung. Plain agar, 5 hours

PLATE 12

FIG. 23. *Bacillus mycoides* from cow dung. Plain agar, 24 hours

FIG. 24. *Bacillus mycoides* from cow dung. Plain agar, 5 days

PLATE 13

FIG. 25. *Bacillus cereus* from milk. Plain agar, 7 hours

FIG. 26. *Bacillus cereus* from milk. Plain agar, 24 hours

PLATE 14

FIG. 27. *Bacillus cereus* from milk. Glucose agar, 24 hours

FIG. 28. *Bacillus albolactus* from milk (*Bacillus lactis albus*). Plain agar, 7 hours

PLATE 15

FIG. 29. *Bacillus albolactus* from milk (*Bacillus lactis albus*). Plain agar plate, 24 hours

FIG. 30. *Bacillus albolactus* from milk (*Bacillus lactis albus*). Glucose agar, 24 hours

PLATE 16

FIG. 31. *Bacillus megatherium* from American Museum. Plain agar, 7 hours

FIG. 32. *Bacillus megatherium* from American Museum. Plain agar (plate), 24 hours

PLATE 17

FIG. 33. *Bacillus megatherium* from American Museum. Glucose agar, 24 hours

FIG. 34. *Bacillus megatherium* from American Museum. Glucose agar, 48 hours

PLATE 18

FIG. 35. *Bacillus megatherium* from Kral. Plain agar, 7 hours

FIG. 36. *Bacillus megatherium* from Kral. Plain agar, 24 hours

PLATE 19

FIG. 37. *Bacillus megatherium* from Kral. Glucose agar, 20 hours

FIG. 38. *Bacillus petasites* from milk. Plain agar, 7 hours

PLATE 20

FIG. 39. *Bacillus petasites* from milk. Plain agar, 20 hours

FIG. 40. *Bacillus petasites* from milk. Plain agar, 48 hours

PLATE 21

FIG. 41. *Bacillus petasites* from Kral. Plain agar, 7 hours

FIG. 42. *Bacillus petasites* from Kral. Plain agar, 20 hours

PLATE 22

FIG. 43. *Bacillus graveolens* from Kral. Plain agar, 7 hours

FIG. 44. *Bacillus graveolens* from Kral. Plain agar, 20 hours

PLATE 23

FIG. 45. *Bacillus tumescens* from Kral. Plain agar, 7 hours

FIG. 46. *Bacillus tumescens* from Kral. Plain agar 20 hours

PLATE 24

FIG. 47. *Bacillus tumescens* from Kral. Glucose agar, 48 hours

FIG. 48. *Bacillus fusiformis* from dust. Plain agar, 24 hours

PLATE 25

FIG. 49. *Bacillus fusiformis* from dust. Plain agar, 48 hours, showing long threads

FIG. 50. *Bacillus fusiformis* from contaminated hirudin. Plain agar, 24 hours

PLATE 26

FIG. 51. *Bacillus fusiformis* from contaminated hirudin. Plain agar, 48 hours

FIG. 52. *Bacillus terminalis* from milk. Plain agar, 17 days

FIG. 1

FIG. 2

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 3

FIG. 4

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 5

FIG. 6

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 7

FIG. 8

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 9

FIG. 10

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 11

FIG. 12

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 13

FIG. 14

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 15

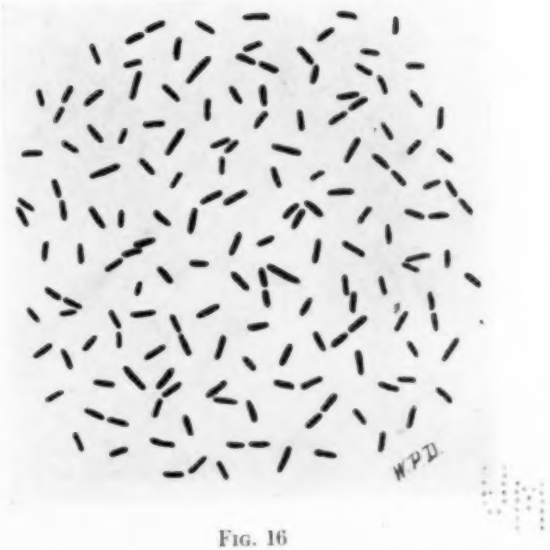

FIG. 16

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

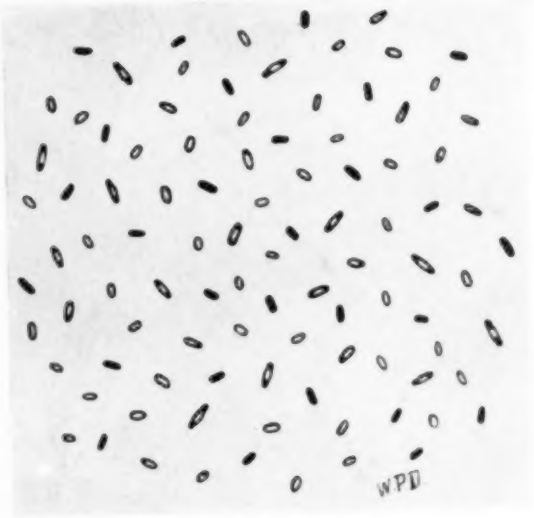

FIG. 17

FIG. 18

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 19

FIG. 20

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 21

FIG. 22

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 23

FIG. 24

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 25

FIG. 26

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 27

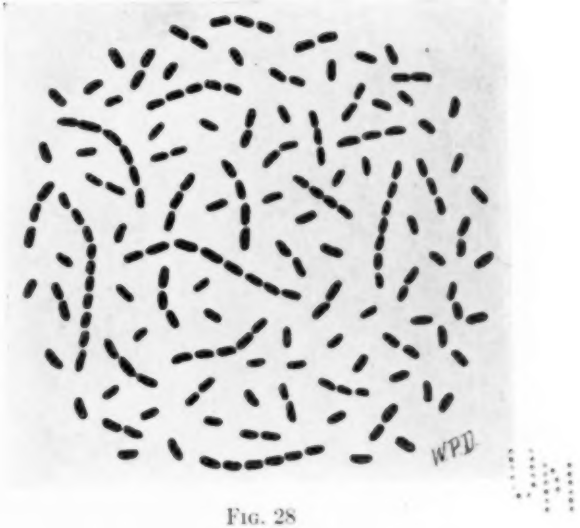

FIG. 28

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 29

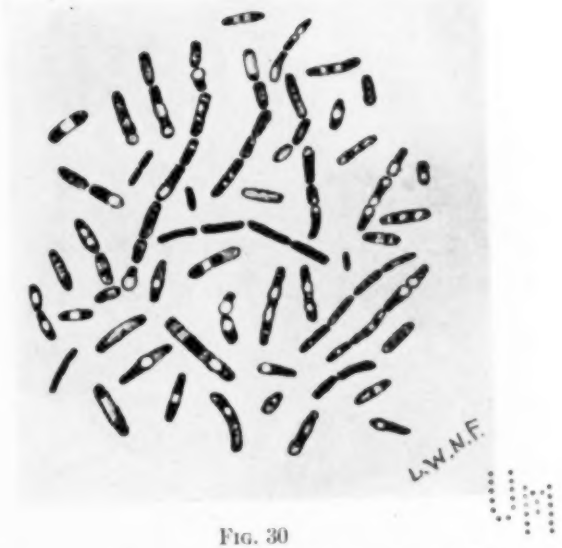

FIG. 30

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

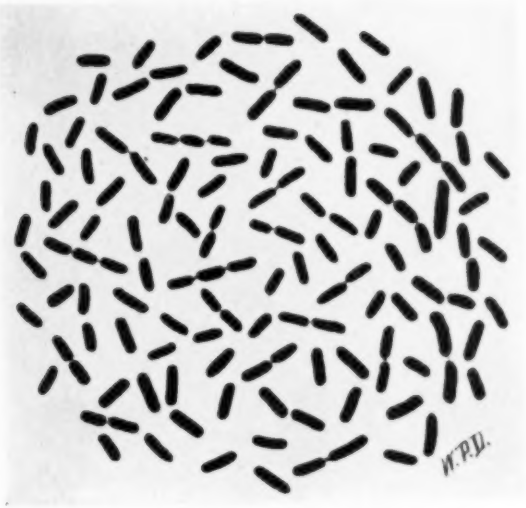

FIG. 31

FIG. 32

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 33

FIG. 34

Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 35

FIG. 36

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria

FIG. 37

FIG. 38

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 39

FIG. 40

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 41

FIG. 42

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

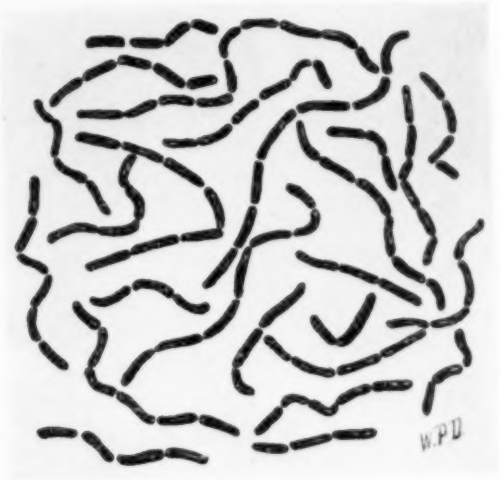

FIG. 43

FIG. 44

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 45

FIG. 46

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 47

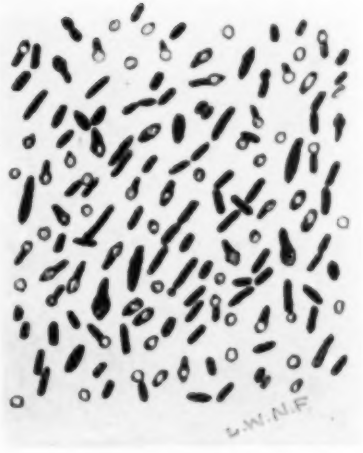

FIG. 48

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 49

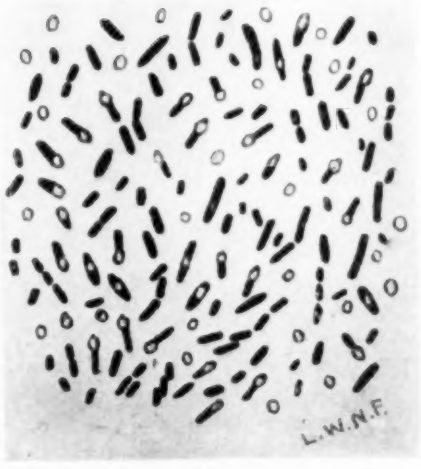

FIG. 50

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria

FIG. 51

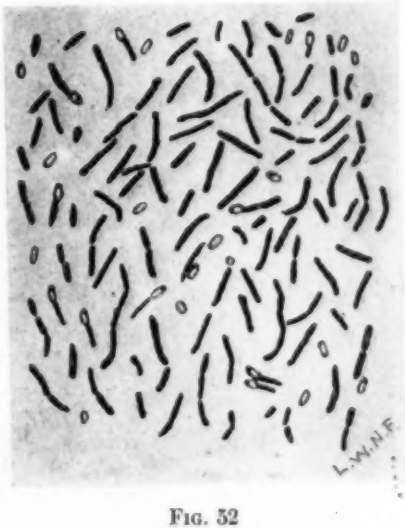

FIG. 52

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

THE NUMBER OF COLONIES ALLOWABLE ON SATISFACTORY AGAR PLATES

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INTRODUCTION

A point which is of much importance in making bacteriological counts is the limit in the number of colonies that may be allowed to grow on a plate without introducing serious errors. Probably every bacteriological worker has this point in mind in making counts and has his own opinion based on experience; but there are few published data on the subject. The matter has been specially under discussion in connection with the proposed revision of standard methods of milk analysis. This investigation was made in order to increase the amount of information available for the use of the Committees who have undertaken the work of revision.

HISTORICAL

It is interesting to note the published opinions of different workers on this point. In 1895 Neisser (1895) published an article in which he reached the conclusion that plates should be so made that they will have about 10,000 colonies per plate, which numbers should then be estimated by means of the low power lenses of a compound microscope. He undoubtedly believed that each bacterial cell put into an agar plate would produce a colony regardless of overcrowding. Three years later Hesse and Niedner, (1898) realizing, to some extent at

[1] The senior author of this paper is responsible for the original suggestion of this problem, for direction in carrying it out, and aid in preparing the esults for publication. The junior author has carried out the laboratory work and has helped in preparing the results for publication.

least, the true state of affairs published an article in which they claim that plates having more than 100 colonies should be disregarded and that under these conditions the microscope should not be used for counting. In 1897, Hill (Hill and Ellms, 1897) contended that overcrowded plates would not give reliable results in water analysis. In 1899, Jordan and Irons (1899) independently urged the same thing. Again Hill (1908) called attention to the point in a paper read before the laboratory section of the American Public Health Association in 1907, in which he pointed out that wide discrepancies in counts might be caused by different methods of computation and concluded that only those plates having numbers of colonies falling between 40 and 200 per plate should be considered in reporting results. These figures were adopted in the report presented by the Committee on Standard Methods for the Bacterial Examination of Milk at the Richmond meeting of the American Public Health Association (1910). In the Report presented at the Rochester meeting in September 1915 (Comm. Stand. Meth. 1915) the lower limit in the number of colonies allowable on agar plates was changed from 40 to 30, and the limits of 30 and 200 were also accepted by the Committee on Standard Methods of Bacterial Water Analysis in their Report presented at the same meeting.

STATEMENT OF PROBLEM

It is generally recognized that the kind of bacteria present in the material under examination will have an influence on the size of the colonies, and, consequently, on the number that can develop on a plate. Some of the commonest and most important bacteria in milk do not produce colonies larger than pin points on ordinary agars even when only a few are present. Other colonies grow large and in the case of spreaders may cover the entire plate.

Just what prevents the development of all the bacteria into colonies on crowded plates is not thoroughly understood. In some cases it may be because the food material is all used up;

in others it is clearly due to the fact that by-products of bacterial growth inhibit the growth of other colonies; and occasionally colonies fuse or overgrow each other and so reduce the count. On the other hand colonies growing side by side sometimes stimulate each other, a phenomenon which has been noted in this work on plates containing large numbers of *B. bulgaricus* with an occasional mold or bacterial colony of a different type. The molds and many bacteria so stimulate the *B. bulgaricus* that these organisms form visible colonies in the region of the larger colonies, failing to develop in all other parts of the plate. The same condition has been noted in plating material containing large numbers of long chained streptococci. This phenomenon naturally produces marked irregularities in count when it occurs.

Because of these and other difficulties certain plates in any series made from a given sample are more satisfactory for use in computing a total count than are others. The matter of selecting plates to be used in computing a count becomes therefore a matter requiring considerable judgment.

EXPERIMENTAL DATA

a. Analyses made in the Station Laboratory

The object of this study has been to determine the limits in the number of colonies on plates which are satisfactory for making bacterial counts. The data used have been obtained by plating market milk samples on standard agar in triplicate and in three different dilutions, incubating for five days at 21°C., following with an incubation for two days at 37°C. The plates were counted at the end of five days and again after the two days incubation at 37°C. The five day and seven day counts are tabulated separately and show the conditions for each period of incubation.

In deciding which plate counts to select as probably nearest correct it became necessary to discard all of the counts on a few samples where no satisfactory average could be made because of spreaders or because the milk contained more bacteria

than was anticipated and the dilutions were not carried far enough to give assurance that the count was not affected by overcrowding. In selecting individual plate counts which were to be tabulated as satisfactory, those counts were chosen which could be used in making an average without any individual figure varying more than 20 per cent from the average. All others were listed as discrepancies. For example, one sample gave the following counts per plate, 1: 100 dilution 1944, 1472 and 1928 colonies; 1: 1000 dilution 484, 515 and 610 colonies; 1: 10000 dilution 43, 45, and 46 colonies. The counts of 484 and 515 from the 1: 1000 dilution were averaged with the 1: 10000 counts of 43, 45 and 46; and this average was taken as the final count on the sample. The counts made on the 1: 100 plates were all listed as discrepancies because they are more than 20 per cent lower than the average, and the count of 610 from one of the 1: 1000 plates was also listed as a discrepancy because it was more than 20 per cent higher than the average. Occasionally all of the nine plates made from a sample could be included in the final average.

Table I gives the number of plate counts made after five days of incubation at 21°C., arranged in groups according to the number of colonies which appeared on the plates. Four hundred and thirty-nine of the 1435 plates had less than 10 colonies per plate. Only 22.3 per cent of these checked within the 20 per cent limit. One hundred and eighty plates fell in the group having more than 10 and less than 20 colonies per plate. Of these 53.9 per cent checked within the 20 per cent limit. Percentages calculated for the groups of plates having 20 to 30, 30 to 50, 50 to 100, 100 to 200 and 200 to 400 colonies per plate were more or less variable, showing that from 66.3 per cent to 93.2 per cent of the total number of plates agreed within the 20 per cent limit. The best percentage of agreement is shown by the group having more than 100 and less than 200 colonies per plate, and the next highest by the group having between 50 and 100 colonies per plate. There were decidedly fewer plates giving satisfactory results among those which had more than 400 colonies per plate, the percentage of plates which checked within 20 per cent being 44.4.

The results given in the lower part of table 1 were calculated from the same counts, the groups of plates having been arranged differently. From this part of the table it will be seen that the percentage of discrepant plates is practically the same for the groups of plates having 20 to 400, 30 to 400, 20 to 200, 30 to 200, or 40 to 200 colonies per plate, the best showing being made by the group of plates having more than 40 and less than

TABLE 1

Plate counts after incubation at 21° C. arranged to show the number and percentage of counts in groups according to the number of colonies per plate

GROUP	CHECKED WITHIN 20 PER CENT OF AVERAGE		DISCREPANT PLATES, DID NOT CHECK WITHIN 20 PER CENT OF AVERAGE				TOTAL NUMBER OF PLATES IN GROUP
	Number	Per cent	Too low	Too high	Total number	Per cent	
0 to 10	98	22.3	172	169	341	77.7	439
10 to 20	97	53.9	29	54	83	46.1	180
20 to 30	54	72.9	6	14	20	27.1	74
30 to 50	67	66.3	11	23	34	33.7	101
50 to 100	162	84.8	17	12	29	15.2	191
100 to 200	179	93.2	8	5	13	6.8	192
200 to 400	105	78.9	25	3	28	21.1	133
Over 400	100	44.4	114	11	125	55.6	225
0 to 30	249	35.9	207	237	444	64.1	693
20 to 400	567	82.0	67	57	124	18.0	691
30 to 400	513	83.1	61	43	104	16.9	617
20 to 200	470	82.9	43	54	97	17.1	567
30 to 200	416	84.3	37	40	77	15.7	493
40 to 200	376	86.0	23	28	61	14.0	437
Over 400	100	44.4	114	11	125	55.6	225

Total number of counts summarized in this table 1435.

200 colonies per plate. Plates having less than 30 colonies or more than 400 colonies show very large percentages of discrepancies.

Table 2 gives the results obtained by counting 1056 of the same plates as those whose counts are summarized in table 1 after two days of additional incubation at 37°C. In general the results obtained from these counts are similar to those given in table 1. However the best showings are made in this case by groups of plates having more than 200 and less than 400 colo-

nies per plate (87 per cent of satisfactory plates), the group of plates having 100 to 200 colonies (82.4 per cent) and the group having 30 to 400 colonies per plate (81.4 per cent). As in table 1 there is a marked increase in the number of discrepant counts from plates having less than 30 or more than 400 colonies per plate While the results in table 1 favor the 40 to 200 group rather than the 30 to 400 group by 2.9 per cent., the same

TABLE 2

Plate counts after two additional days of incubation at 37° C. arranged to show the number and percentage of counts in groups according to the number of colonies per plate

GROUP	CHECKED WITHIN 20 PER CENT OF AVERAGE		DISCREPANT PLATES, DID NOT CHECK WITHIN 20 PER CENT OF AVERAGE				TOTAL NUMBER OF PLATES IN GROUP
	Number	Per cent	Too low	Too high	Total number	Per cent	
0 to 10	60	28.4	60	91	151	71.6	211
10 to 20	76	60.0	23	28	51	40.0	127
20 to 30	46	63.0	8	19	27	37.0	73
30 to 50	55	72.3	5	16	21	27.7	76
50 to 100	117	81.0	14	12	26	19.0	143
100 to 200	127	82.4	16	11	27	17.6	154
200 to 400	101	87	14	1	15	13	116
Over 400	78	50	74	4	78	50	156
0 to 30	182	44.2	91	138	229	55.8	411
20 to 400	445	79.2	57	61	117	20.8	562
30 to 400	399	81.4	49	42	91	18.6	490
20 to 200	353	77	45	60	105	23	458
30 to 200	307	79.7	37	41	78	20.3	385
40 to 200	277	79.8	36	34	70	20.2	347
Over 400	78	50	74	4	78	50	156

Total number of counts summarized in this table 1056.

comparison in table 2 shows an advantage of 1.6 per cent for the 30 to 400 group This indicates that there is little advantage in selecting one group of plates in preference to the other.

In the fourth and fifth columns of these two tables, the number of cases is shown in which the discrepancy was caused by having too few or too many colonies on the plate Arranging the plates in the groups 0 to 10, 10 to 20, 20 to 30, 30 to 50, 50 to 100, 100 to 200, 200 to 400 and more than 400 colonies per

plate, it is seen that there is a tendency for discrepancies caused by having too many colonies on a plate to occur in all groups having less than 50 colonies per plate (one exception to this statement is seen in the group 0 to 10 in table 1). In all cases where more than 50 colonies occurred on the plates, the greater number of discrepancies was caused by having too few colonies on the plates. The tendency toward discrepancies caused by having too few colonies on the plates becomes very marked as soon as the limit of 200 colonies per plate is passed.

These findings indicate that while the greater proportion of the discrepancies on plates having less than 50 colonies per plate are caused by the operations of the laws of choice and chance, yet there is some factor present which tends to cause more colonies to develop than should do so. In all probability this factor is chance contamination from the air which occurs during planting. As is well known, it is common for supposedly sterile check plates to develop one, two or more colonies on prolonged incubation. The presence of these colonies on inoculated plates having fewer than 50 colonies per plate causes a relatively large error in the counts which in some cases would cause the individual plate count to exceed the 20 per cent limit specified here as necessary before the plates were classed as satisfactory.

The tendency for irregularities, due to having too few colonies on plates, to occur in counts having 50 or more colonies per plate is too well known to all bacteriologists to require extended discussion. These are undoubtedly caused by the effect of overcrowding. The fact that not all of the discrepancies on plates having more than 400 colonies per plate were of this sort is more significant, for it shows that not all of the discrepancies on plates having numerous colonies are due to overcrowding. Irregularities in the number of bacteria used in inoculating or chance contaminations are two things which might produce plates having too many colonies even on crowded plates.

When all of these things are taken into consideration, it becomes a difficult matter to decide upon the limits in number of colonies which should be allowed on plates. It is at once

clear that plates having less than 20 and more than 400 colonies are so apt to be widely discrepant that counts from plates of this sort should be disregarded. There are likewise clear indications that plates having between 40 and 200 colonies per plate are as satisfactory as any that can be selected. However the results secured in this investigation do not indicate that serious errors would be introduced in routine work by extending these limits to 30 and 400, or even to 20 and 400, thereby lessening the amount of work necessary to secure acceptable counts.

b. New York City analyses

Another set of data which is more satisfactory in one way because of the fact that a very large number of plates were made from a single sample of milk but which is also less satisfactory in another way because of the fact that it is more limited in its application, has been secured from a set of analyses made on November 19, 1915, by five New York State laboratories,[2] under the supervision of Prof. H. W. Conn. In this series 20 samples of the same milk were sent to each laboratory for analysis. Four laboratories made plate counts, one making them in duplicate, so that five sets of plate counts are available. These were made from two dilutions of 1: 100 and 1: 1000 each. Two plates were made for each dilution. Three laboratories made microscopic counts, one making them in duplicate so that four sets of these counts are available.

The average of the accepted plate counts was 4250. The average of the microscopic counts of clumps, or sources, was 5590. The close correspondence in results obtained by these two very different methods of counting makes it very probable that the total number of groups of bacteria in this milk was close to 5000 per cubic centimeter. The 1: 100 dilution plates gave counts in which the average number of colonies on the two plates varied between 24 and 125. The 1: 1000 plates gave counts in which the average number of colonies from the two plates varied be-

[2] Lederle Laboratories, North's Sanitary Laboratories, N. Y. City Board of Health Laboratory, Borden's Laboratory, N. Y. Agric. Exp. Sta. Laboratory.

tween 0.5 and 16.5 with a single case where the average of the two plates was 44.

If we arbitrarily assume that plates giving a count more than 2500 above or below the average fail to check with the accepted count, we find that the averages of all but three of the 100 pairs of 1: 100 plates check with the accepted count while there are 27 cases out of the 100 where the count from the 1: 1000 dilution fails to check within these limits. It is important to note also that 23 of these 27 cases are instances where the discrepancy was such as to give a higher count that the accepted count, indicating that chance contaminations were probably the chief cause of trouble.

SUMMARY

1. The work here reported includes a study of the counts made from 1435 agar plates inoculated from samples of market milk and incubated five days at 21°C.; and also a study of the counts made from 1056 of the same plates after two days additional incubation at 37°C. The results obtained indicate that, for milk analyses, the counts made from plates having more than 30 and less than 400 colonies on the plates are very nearly as satisfactory as those obtained from plates having more than 40 and less than 200 colonies, the latter being the limits in numbers originally recommended by the Committee on Standard Methods for the Bacterial Examination of Milk.

2. Plates having less than 20 or more than 400 colonies on them are shown to be so frequently discrepant that counts obtained from them should never be trusted unless checked by comparison with plates from different dilutions having more than 30 or less than 400 colonies. The acceptance of counts from plates having 20 to 30 colonies per plate would not greatly increase the percentage of discrepancies.

3. All groups of plates, regardless of the number of colonies showed a certain percentage of plates which gave counts which varied more than 20 per cent from the accepted count. The percentage of discrepant counts of this sort varied between 37

and 7 for all groups of plates having more than 20 and less than 400 colonies per plate, the worst showing being made by the plates having 20 to 30 colonies per plate and the best by the plates having 100 to 200 colonies per plate.

4. The discrepancies which occurred in counts made from plates having less than 50 colonies per plate were more frequently caused by too many colonies on the plates than by too few colonies. This excess is undoubtedly due to the influence of chance air contaminations which took place during the plating. Where the plates have a small number of colonies on them a few extra colonies of this sort produce relatively wide discrepancies.

5. The discrepancies in counts made from plates having more than 50 colonies per plate were more frequently caused by having too few rather than too many colonies on the plates. The frequency of this type of discrepancy became very marked where the number of colonies exceeded 200 per plate. The probable explanation of the excess of this type of irregularity is that of overcrowding. Since however there was always a certain percentage of discrepancies caused by having too many colonies on the plate even where there were more than 400 colonies per plate, it is evident that not all of the irregularities are caused in this way.

6. Counts made from 20 duplicate samples of the same milk in five series of analyses showed 27 out of a possible 100 wide discrepancies in the counts obtained from an average of two plates made from a 1: 1000 dilution. The number of colonies of these plates averaged more than 0.5 and less than 16.5 for the two plates, with one exception where the average was 44. Counts made from the 100 pairs of 1: 100 plates which had more than 24 and less than 125 colonies as the average of the two plates, showed only 3 out of a possible 100 wide discrepancies.

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A MODIFICATION OF THE HYGIENIC LABORATORY METHOD FOR THE PRODUCTION OF TETANUS TOXIN

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In the November, 1915, issue of the Journal of Medical Research,[1] Anderson and Leake briefly describe the method used at the Hygienic Laboratory, Washington, D. C., for the production of a uniformly potent toxin. The method in use at the Research Laboratory is essentially that given by Anderson and Leake with a few slight variations. As there have been many inquiries as to how we obtain our potent toxin it was thought that the details of the exact procedure might be of sufficient value to publish.

STOCK CULTURES

The stock cultures are grown on a semi-solid medium made in the following way.

Veal broth	1000 cc.
Agar	5 grams
Witte's Peptone	10 grams
NaCl	5 grams
Reaction	Neutral to phenolphthalein

About 8 cc. to 10 cc. of this medium are put into tubes which are autoclaved at 15 pounds pressure for one-half hour, the tubes being then ready for use.

To transfer cultures, one of the semi-solid agar cultures is melted and 1 cc. is added to a freshly melted semi-solid agar tube, at least ten sub-cultures being thus made from one stock culture. After inoculation, the tubes are cooled, the plugs immersed in

[1] Anderson and Leake, 1915. A Method of producing Tetanus Toxin, Journal of Medical Research, **33**, 239.

paraffin and the tubes incubated at 37°C. After one week's incubation the cultures are stored in the ice chest, where they may be kept for six months without affecting their ability to produce toxin.

THE BROTH FOR TOXIN AND PRELIMINARY CULTURES

The following broth is used both for the toxin and for the preliminary cultures for inoculating the toxin broth:

To every pound of market veal, add 1000 cc. of water and place in ice chest over night. The next morning the infusion is placed over a free flame and raised to 45°C. and held at that temperature for one hour; it is then boiled briskly for one-half hour and the broth strained through cheesecloth. The amount of filtrate is measured and the following added:

Witte's Peptone	1.0 per cent
Glucose (anhydrous)	1.0 per cent
NaCl	0.5 per cent

When the above ingredients are added, the broth is boiled until the peptone, glucose, etc., are melted, and is then titrated with $\frac{N}{10}$ NaOH. After the reaction has been corrected to +1 to phenolphthalein, the flame is turned out. The broth is then filtered through two layers of absorbent cotton directly into 2 litre Erlenmeyer flasks, leaving only sufficient space in the flasks for the expansion of the broth during the sterilization in the Arnold. The flasks are sterilized for $1\frac{1}{2}$ hours on the first day and one hour on the second day.

PRELIMINARY CULTIVATION

Fill potato tubes with about 40 cc. of the glucose broth and sterilize for one and one-half hours on the first day and one hour on the second day. These tubes may be kept for two weeks when they will still give satisfactory growth. To make the first transfer for the preliminary cultivation, add 8 cc. of the melted semi-solid agar stock culture of the *B tetani* to two tubes of glucose broth from which the air has been previously

expelled by heating in the Arnold for fifteen to twenty minutes, and which have been cooled down to about 50°C. These tubes are incubated for 24 hours and the next day, two freshly heated tubes of glucose broth are inoculated with 5 cc. of the glucose broth cultures planted the previous day. On the third day, determine the number of flasks that are to be inoculated and inoculate as many freshly heated glucose broth tubes from the second glucose broth generation as there are flasks. Anderson calls for at least six or seven generations in the glucose broth before the inoculation of the toxin broth, but at the Research Laboratory it has been found that three generations or even two if need be, are sufficient for obtaining a toxicity of 1–25,000.

INOCULATION OF TOXIN BROTH

After the second sterilization in the Arnold, the flasks are ready for inoculation. The broth may be cooled down to 55°–60°C. by allowing the flasks to stand at room temperature, or in a more rapid way by placing the hot flasks in a large sink, to which cool, and then cold, water is added until the lower portions of the flasks are covered. When the bottoms of the flasks are cool to the hand, the portions above the water being still very hot, the inoculation may be made as follows:

The plugs are carefully removed, the necks flamed and the plugs replaced. In a similar way, the mouths of the culture tubes are sterilized and then, partly removing the plug of a flask, the contents of a potato tube is poured rapidly into a flask. If one prefers, the broth culture may be transferred by using a pipette, but the former method has been used here without subsequent contamination and found very satisfactory. After inoculation, the flasks are incubated for fifteen days at 36°–37°C., care being taken to exclude all light from them.

The flasks at the end of 24–48 hours show a diffuse cloudiness with the formation of gas bubbles on the surface of the broth. Toward the end of two weeks, the gas bubbles usually disappear, while the cloudiness persists and a light precipitate forms at the bottoms of the flasks. If it is not convenient

to filter on the fifteenth day, the cultures may be kept in the incubator until the twentieth day without a loss of toxicity but from the twentieth day to the twenty-fifth day, the toxin loses about 20 per cent in potency.

FILTRATION

All glassware, filters, etc., should be neutral to phenolphthalein and the greatest care should be taken to exclude light, either direct or indirect, by darkening the room and by covering the filtering apparatus with dark cloths, ordinary black cambric being used at the Research Laboratory.

The broth cultures are first passed through Buchner filters about 8 inches in diameter, which have been packed with a layer of finely shredded paper pulp 0.25 inch in thickness. It is of importance that the pulp should be so well packed that the filtrate is absolutely clear, otherwise it will clog the Berkefeld filter. The first filtrate, about 200 cc., which passes through the Buchner is discarded, as it contains a considerable amount of water from the pulp, and then the filtering of the toxin may proceed. If, after passing 8 to 14 litres through the pulp, the filtrate begins to appear cloudy, the pulp must be discarded and the Buchner repacked. The clear filtrate is then passed through a sterile Berkefeld filter, and 10 per cent of a 5 per cent solution of carbolic acid solution is added to the toxin which is now placed in the ice-chest, ready for testing its potency.

POTENCY TEST

Two 350 gram guinea pigs are inoculated subcutaneously over the abdomen with 1 cc. of a dilution of $\frac{1}{15,000}$ and $\frac{1}{25,000}$ of the toxin respectively. If the toxin has a potency of $\frac{1}{25,000}$ the pig receiving the $\frac{1}{15,000}$ dilution will die on the second to third day and the pig receiving the $\frac{1}{25,000}$ dilution should die on the fourth day. If both pigs die with symptoms of tetanus before the fourth day, the toxin is stronger than $\frac{1}{25,000}$ and a higher dilution should be tested.

Though no comparative tests have been made with toxin produced under the usual anaerobic conditions, the toxin produced by the Hygienic Laboratory method showed a somewhat unexpected stability. For example:

	Filtered	*Tested*	*Toxicity*
Lot 25	7-24-14	7-24-14	1-25,000
		8-31-14	1-25,000
		9-30-14	1-20,000
		10- 4-14	1-20,000

The test was made on a small amount of toxin, about 30 cc., kept in an ordinary test tube in the ice-box and protected from the air only by a shallow covering of albolene.

From the above, it will be seen that our method for growing the tetanus bacillus for toxin varies very slightly from that of the Hygienic Laboratory. Instead of beef, we use market veal which has given almost invariably a toxin of $\frac{1}{25,000}$, even going as high as $\frac{1}{40,000}$ occasionally. It is interesting to note that with the bob veal, that is, veal under the legal age limit for selling, which has always given a more highly potent diphtheria toxin than the older veal, we have rarely obtained a tetanus toxin above $\frac{1}{15,000}$ whereas the market veal used as control has produced a toxin of $\frac{1}{25,000}$ strength.[2]

Anderson recommends that the preliminary cultures should be carried on from one to three weeks, by daily transfers, but with only three generations in glucose broth before inoculating the toxin flasks, we have obtained a toxicity of 1–40,000 showing that fewer generations may be sufficient.

Here it might be well to state that we obtained different results from those recorded by other writers in regard to the growth in the preliminary cultures. Anderson and Leake found that a good growth is obtained in the first generation in 48 hours, in 24 hours in the second and third transplants, and in 16 hours

[2] Furthermore, by inoculating the glucose broth directly with a melted semi-solid agar culture of *B. tetani*, we have secured a toxin of over $\frac{1}{25,000}$ in strength, the control test which had been inoculated from preliminary broth cultures giving the same degree of toxicity. Confirmatory tests are being made along this line.

in the fourth. At the Research Laboratory, we have found an abundant growth in 18 to 24 hours in the first generation in glucose broth from the semi-solid; this profuse growth continues in the subsequent cultures up to the third or fourth generations, when there is a diminution, until in the sixth or seventh, as frequently happened, no growth or very slight growth appears after 48 hours or more. That is, out of thirteen cultures transferred in the sixth generations, there may be only six or seven tubes which show signs of growth, even after several days incubation.

At the Hygienic Laboratory, glucose stab cultures are used for growing the stock strains, but it is not stated whether these cultures are grown anaerobically or aerobically. The semi-solid agar that we are using for the stock cultures is especially satisfactory as no anaerobic conditions except such as the medium provides are necessary to produce a heavy growth of *B. tetani* with spore formation after a few days incubation.

It is in the hope that other laboratory workers may find the above technique of practical aid in producing an uniformly potent toxin that these minute details have been given.

A METHOD OF ANAEROBIC PLATING PERMITTING OBSERVATION OF GROWTH[1]

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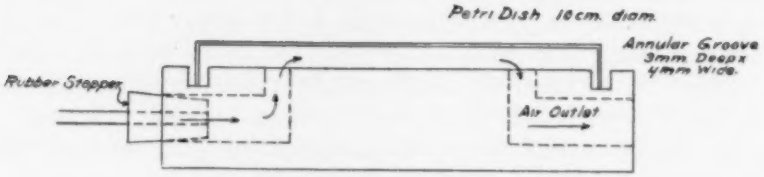
Because of the already numerous descriptions of methods for growing cultures anaerobically, one hesitates to add another method to the list without an apology. The method here described will however be found of advantage in the isolation of anaerobes from cheese, milk, soil or other material where it is desired not only to secure growth of the anaerobes, but also to obtain them in pure culture directly from the material under examination. The method is chiefly to be recommended because of the relatively small amount of inert gases necessary to replace the air in contact with the media—an advantage that will be appreciated in laboratories where generous supplies of these gases are not available and where the method of anaerobic plating is frequently employed on a small scale. Furthermore. the rate and character of growth of the colonies are easily observed from the beginning, so that it is not necessary to open the anaerobic chamber at any time until sub-cultures are to be made.

The apparatus consists of one half of a Petri dish sealed with paraffine, of relatively low melting point, on a square stone or metal base provided with an inlet for the inert gas and an outlet for the displaced air. The base is conveniently made as follows: Slabs of stone (such as Alberine stone, or soapstone) or of cast iron, of about 2 cm. thickness, are cut in squares about 1 cm. larger than the diameter of the Petri dish to be used. Cut in one face of these slabs, with a lathe, an annular groove 3 mm. deep and 4 mm. wide, and of such a diameter that the

[1] Published by permission of the Secretary of Agriculture.

edges of the Petri dish will fit loosely into this groove. Within the circle described by this groove, about 2 cm. from an edge of the slab; i.e., about 5 mm. from the inner edge of the groove, drill a hole about 4 mm. in diameter and about half the thickness of the slab in depth. Drill a similar hole for the opposite side of the annular groove. Now drill horizontal holes from the corresponding surface edges of the slab to meet the vertical holes, and of such a diameter that small rubber stoppers may be used to stopper these holes securely. With this slab as a base for the Petri dish, the method of manipulation, provided it is desired to grow all of the colonies on the surface of the media, is somewhat as follows: Pour the agar or gelatin into the Petri dish as usual and allow to solidify. Then flow suitable water or broth dilutions of the material to be examined on the surface



Cross Section view of stone base with petri dish in position.

of the medium. Tilt the dish to one side, and, with a sterile pipette, withdraw the excess of the diluting fluid. Invert the dish into its sterile cover and allow to drain. This draining will prevent contaminations on the stone base from spreading upward on the plate and so obviate the necessity of sterilizing the base. Now place the dish, edges down, into the annular groove of the slab, which has been previously warmed to a temperature sufficient to melt paraffine with a melting point of say 45 degrees Centigrade. The plate may be sealed to the stone by flowing melted paraffine into the groove either before or after the plate is put into position in the groove. The stone is then allowed to cool until the paraffine is thoroughly congealed. There are now, between the stone and the surface of the medium, only about 20 cc. of air to be replaced by the inert

gas, and by leading the gas from a suitable generator in through one of the rubber stoppers, (allowing the air to escape by way of the opposite loosely-stoppered opening) the oxygen pressure inside the inclosed space may be reduced to less than 1 mm. by the use of only about 200 cc. of the gas. The holes are then securely closed and the plates are ready for incubation.

Oxygen- and CO_2-free air has been secured by the following method: Connect in series one or more of the sealed Petri dishes with two wash bottles containing a 5 per cent pyrogallic acid 10 per cent caustic soda solution. Force air slowly through this train and allow the displaced air to escape by way of the opposite outlet of the sealed Petri dish.

When hydrogen is to be used for displacing the air, two wash bottles are required: One of $AgNO_3$ solution for traces of ASH_3, and one of lead acetate solution for H_2S.

In general, satisfactory anaerobic conditions are obtained when the volume of H_2 or N_2 allowed to pass through the sealed plates amounts to ten times the volume of air inclosed by the plates and their connections.

TESTICULAR INFUSION AGAR—A STERILIZABLE CULTURE MEDIUM FOR THE GONOCOCCUS

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INTRODUCTION

The use of testicular infusion agar suggested by Hirschfelder (1914) aroused the hope that a medium for the cultivation of the gonococcus had been found which might be sterilized by steam, thus avoiding the addition of raw albumin (ascitic fluid or blood) to agar with its uncertain sterility and frequent failure to support growth even when sterile. Unfortunately I cannot agree that by the use of his formula all difficulties in cultivating the gonococcus (at least in pure culture) are removed, as he claims. I have however, determined *some* of the factors affecting successful cultivation of gonococci in comparatively large quantities upon a sterilizable agar containing infusion of testicle.

Vannod (1905) claimed that proper adjustment of the reaction with sodium carbonate facilitated cultivation of the gonococcus on so called ordinary media but the possible variation from the optimum is so slight that the method has not come into general use. One of Vannod's later contributions (1907) testifies to the general acceptance of the idea of the necessity of adding raw albumins.

More recently some success in improving the media has been attained by Schwarz and McNeil (1912) in this country with so called "salt free" veal agar, which is now generally used in the preparation of polyvalent antigens for the alexin fixation test.

[1] The experimental work of this paper was carried out and its practical application made in The Cutter Laboratories, Berkeley, California, and it is published with the consent of the Director, Dr. H. E. Foster.

Abroad, Lumiere and Chevrotier (1913) have advocated a mixture of beer wort and albumin sterilized in the autoclave, to which however, the addition of sterile horse serum is said to be advantageous, though not indispensable. Upon this medium gonococcus cultures were found to be viable at remarkably low temperatures (Lumiere and Chevrotier, 1914).

Emile Weil and Noire (1913) have also suggested an agar containing whey, peptone, saccharose and urea. I have failed in several attempts to corroborate their claim that gonococci would grow upon this medium.

I have not tried the cultivation of gonococci upon the egg broth of Besredka and Jupille (1913) nor according to the method of Ohlmacher (1915) upon Loeffler's blood serum but in several tests upon the starch agar of Vedder (1915) I have found it to be one of the most promising media. However, the growth, while possibly less long lived upon testicular infusion agar, is so much more abundant that the use of the latter is recommended for the preparation of gonococcic vaccine. It should prove equally valuable for the preparation of antigens to be used in the alexin fixation test but this remains to be determined.

CULTURES

My strains of gonococci came originally from clinically typical cases of urethritis and epididymitis, having been isolated upon blood agar and cultivated in some cases as long as two years on ascitic agar. All were typical gram negative biscuit shaped diplococci, showing sparse growth upon rabbit blood or ascitic agar and failure of growth, at least in the second subculture, upon plain agar at 37°C. These have been our criteria and while we have had a realization quickened by the work of Broughton-Alcock (1914) that we might occasionally exclude true gonococci thereby we have not hesitated to insist that our media should be tested particularly on the less saprophytic strains. At the conclusion of each experiment therefore the purity of each culture was checked by gram stain and failure of growth upon plain agar at 37°C.

Torrey's strains "C," "K," "L," "N," "O," and "S" kindly supplied by Dr. Charles Krumwiede, Jr., of the New York City Board of Health, conform to the above requirements and like our own were found to grow abundantly upon testicular infusion agar.

The hope that opportunity might be found for making comparative tests of this medium in the isolation of gonococci from lesions has already deferred publication so long that it seems likely this will have to be left for some one more advantageously situated. Dr. H. E. Foster,[2] has however succeeded sufficiently often in cultivating the gonococcus from cases of gonorrhea to warrant making such comparisons.

EXPERIMENTAL WORK

Successful cultures were first secured with a slightly acid medium comprising aqueous infusion of beef testicle (500 grams per litre of distilled water), 2 per cent Witte's peptone, 2 per cent agar, 0.5 per cent glucose, and 0.3 per cent NaH_2PO_4, nearly neutralized with N/1 NaOH and sterilized by intermittent steaming in the Arnold sterilizer on three successive days for 30 minutes at 100°C.

This formula differs from Hirschfelder's particularly in its sugar content, the advisability of which was shown by Elser and Huntoon (1909) and by Martin (1911). In each succeeding series of experiments a single factor was varied, the control consisting in a combination previously found successful; as the limits of variability of each factor involved were determined the preferable procedure was adopted for the following experiments.

Thus it was quickly shown that sterilization in the autoclave at 10 pounds pressure for 30 minutes is permissible. The substitution of veal for testicle infusion was found to yield a less vigorous growth. The optimum amount of testicle was found to be 500 grams per liter although fair growth resulted when the proportion was as low as 125 grams per liter of water. The

[2] Personal communication.

use of equal parts of veal extract seemed not to decrease the volume of growth but we have adhered to the use of testicle infusion alone.

AGAR

The amount of agar is important as shown in table 1. Media were made from the same testicular infusion in four lots with 1, 2, 3 and 4 per cent agar. After sterilization the slanted tubes were left in the incubator at 37°C. for three days to dry out

TABLE 1

Optimum amount of agar

AGAR %	CULTURE	16 HOURS	40 HOURS*	64 HOURS
1	G2	None	Poor	Slight
	G3	None	None	None
	G5	None	None	None
2	G2	None	Fair	Fair
	G3	None	Fair	Fair
	G5	Slight	Fair	Good
3	G2	Slight	Fair	Good
	G3	Slight	Fair	Good
	G5	Slight	Fair	Good
4	G2	Slight	Fair	Fair
	G3	Slight	None	Excellent
	G5	None	None	Good

* Patchy colonies respread on all tubes.

NOTE. In this and other tables a "slight" growth approximates that of *B. influenzae* upon blood agar; a "fair" growth corresponds to that of *B. typhi* upon plain agar; "good" to that of *B. coli;* and "excellent" to that of *Bact. pneumoneae.*

the surface of the slopes, a point shown to be necessary by ample experience. The slopes were inoculated from 48 hours ascitic agar cultures, incubated at 37°C. and observed daily.

Soft testicular infusion agar has in our hands regularly yielded less satisfactory results than that which was more firm and less moist, a fact apparently at variance with the experience of McCann (1896) working with cyst fluid agar, and Van Saun (1913) with "salt free" veal agar. Firm testicular infusion agar is moreover not only favorable for growth but facilitates

the removal of the gonococci without the admixture of solid particles of medium.

Warden (1915) has recently pointed out that one of the factors in the autolysis of gonococci is excessive moisture; it is suggested also that weak acids may inhibit autolytic disintegration. At any rate smears from testicular infusion agar cultures contain more whole cocci than those from ascitic agar, but whether the acid reaction due to fermentation of the glucose or the freedom of the media from excessive moisture, or both, may be held responsible in this case can not be stated with certainty. However, the factor of moisture in the media had to be reckoned with in all our experimental and practical work so that frequently where a clear cut result could not be obtained with fresh media there was little difficulty after a few days drying.

I have chosen 3 per cent agar as the most suitable for further use.

GLUCOSE

In the preparation of a portion of one lot of medium the usual glucose was omitted. The prepared slants were dried at 37°C. for 48 hours and afterwards at room temperature for four days. The media still appeared quite moist and the growths upon the controls where they appeared at all were patchy and unsatisfactory even on further incubation after respreading. Further drying at room temperature for ten days however led to a satisfactory result as shown in table 2, cultures being made from 24 hour ascitic agar slants and incubated at 37°C.

TABLE 2

Omission of glucose

MEDIA	CULTURE	16 HOURS	40 HOURS	60 HOURS
With added glucose, 0.5 per cent	G1	Good	Excellent	Excellent
	G2	Good	Excellent	Excellent
	G3	Good	Excellent	Excellent
Without added glucose	G1	None	Slight	Slight
	G2	Fair	Good	Good
	G3	Fair	Fair	Good

The usual control tests failed to show contamination in any tube so that the above result demonstrates that growth is possible without added glucose but is not so good as with it.

That a modicum of carbohydrate is necessary is indicated by a lot of media made as usual with the exception of added glucose and from which only a portion[3] of the tissue carbohydrates had been removed by the growth of *B. communior*, in which no growth of gonococci could be secured. But media which had been so fermented and then re-inforced by addition of 1 to 2 per cent glucose yielded very excellent growth showing that inhibition in the fermented media could scarcely have been due to the accumulation of metabolic wastes from *B. communior*. Thus it was shown that for these strains the order of preference for added glucose content in media previously fermented by *B. communior* is 1 per cent, 2 per cent, and 3 per cent. Since, however, I have found no advantage in a preliminary fermentation the addition of 0.5 per cent glucose to unfermented media has been retained.

PHOSPHATES

The use of unsaturated phosphates in culture media for bacteria was recommended by Henderson and Webster (1907) for their stabilizing effect upon the reaction, and a medium of this sort plus human serum was advocated by Martin (1911) for the cultivation of the gonococcus.

My experiments upon the *necessity* of *added* phosphate have been inconclusive; at times excellent growth has been secured without its addition. In three separate double lots of testicular infusion agar made with and without the addition of inorganic phosphate the advantage has been in favor of that containing it. I have made no effort to determine the optimum amount or to attempt the cultivation of gonococcus in phosphate free media.

[3] That the tissue sugar was not completely eliminated was proven by further gas production in deep tubes of the supposedly sugar free testicular infusion agar by *B. communior*.

REACTION

The inclusion of 0.3 per cent NaH_2PO_4 permits a considerable variation in the amount of sodium hydroxide added to reduce the titrable acidity. The results of a typical controlled experiment upon this point are shown in table 3. I might mention here having previously encountered some difficulty in the addition of

TABLE 3

Range of reaction of testicular infusion agar

CUBIC CENTIMETERS N/1 Na OH ADDED IN 110 CC.	TITRE		CULTURE	16 HOURS	40 HOURS	64 HOURS
	Hypothetical end point*	Actual end point				
0	+6.0	-6.0	G1	None	None	None
			G3	None	None	None
			G5	None	None	None
2	+4.0	+4.2	G1	None	None	Slight
			G3	Slight	Fair	Good
			G5	Slight	Fair	Good
4	+2.0	+3.2	G1	Fair	Excellent	Excellent
			G3	Fair	Excellent	Excellent
			G5	Good	Excellent	Excellent
6	0	+1.7	G1	Good	Excellent	Excellent
			G3	Fair	Excellent	Excellent
			G5	Good	Excellent	Excellent
2	−2.0	?†	G1	Slight	Good	Excellent
			G3	Slight	Good	Excellent
			G5	Fair	Good	Excellent
10	−4.0	Alkaline †	G1	None	None	None
			G3	None	None	None
			G5	None	None	None

*Assuming no unsaturated compounds.

† Media darkened by caramelization—end point uncertain.

more than sufficient alkali to saturate the phosphate, the glucose being thereby caramelized on heating with resultant inhibition of growth of the gonococcus.

A liter of medium was prepared with the usual testicular infusion, 2 per cent peptone, 3 per cent agar, 0.5 per cent glucose and 0.3 per cent Na H_2PO_4. Before neutralization 5 cc. titrated hot with phenolphthalein required 6 cc. N/20 NaOH to show

color. Six lots of 100 cc. each were separated and to each was added the amount of N/1 NaOH shown in table 3, and the total volume of each lot was then equalized at 110 cc. by addition of distilled water. The various media were tubed, sterilized in the autoclave at 10 pounds for 30 minutes, slanted, and dried at 37° C. for six days. Inoculation was made from 24 hour testicular infusion agar cultures; incubation was at 37°C. and the usual control tests confirmed the purity of the growth observed.

It may be seen that "excellent" results may be expected between the limits of actual titre to phenolphthalein from below +1.7 to +3.2 Normal acidity. There will be found little if any difficulty in the reaction when adjustment is made by addition of N/1 NaOH as if the acidity were to be reduced to a titre of zero. It will still be found sufficiently acid, thanks to the phosphate, to obviate the difficulty of caramelization.

PEPTONE

The recent scarcity of Witte's peptone has necessitated experiments upon the substitution of an American product. These have shown "Difco" peptone of the American Digestive Ferments Co., Detroit, Michigan, to be equal to Witte's for this purpose.

VIABILITY

In contrast with the experience of Vedder (1915) with starch agar, prolonged viability cannot be claimed for cultures of gonococci upon testicular infusion agar. It was found that daily transfer of several strains for two weeks was eminently successful; planting every other day failed to keep some of the strains alive for more than four transfers and in all of these there was evidence of deterioration.

Inoculating testicular infusion agar as well as ascitic agar, starch agar, "salt free" veal agar or blood agar from another medium we have often found it necessary to coax the growth by repeated transfers and especially by respreading, before the maximum crop could be obtained. For this reason much emphasis should be placed upon the importance of personal experi-

ence in handling gonococcus cultures. I am pleased to thank Miss Vera Bennett who has prepared much of the culture media for me and Miss Lettie Watkins who has assisted in keeping the stock cultures alive and planting the experimental media.

SUMMARY

The formula now followed in preparing testicular infusion agar for the growth of the gonococcus in preparing suitable vaccines.

1. Mix 500 grams ground beef testicle from which the tunica vaginalis has first been stripped, with 1000 cc. distilled water.
2. Soak overnight at room temperature.
3. Heat to 50°C. Keep warm for one hour by placing in the incubator at 37°C.
4. Boil, strain, and restore to 1000 cc. with distilled water. If in excess do not reduce by boiling since overheating is injurious.
5. Add 2 per cent peptone (Witte's or Difco), 3 per cent agar chopped fine, 0.5 per cent glucose, 0.3 per cent NaH_2PO_4.
6. Soak at least one hour to soften the agar.
7. Melt in the autoclave at 10 pounds pressure for 30 minutes.
8. Titrate with phenolphthalein and add N/1 NaOH sufficient to neutralize if no unsaturated compounds were present.
9. Check the titre by repetition. 5 cc. should require from 1.0 to 2.0 cc. N/20 NaOH to display color hot.
10. Tube and sterilize in the autoclave at 10 pounds for 30 minutes.
11. Slant or pour into plates.

This medium may be melted for plating, etc., but the less heating the better. Filtration for the purpose of removing the distinct turbidity of the medium also seems to be a disadvantage.

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BOOK REVIEW

Der Erreger der Maul—und Klauenseuche. By DR. HEINRICH STAUFFACHER. Wilhelm Engelmann, Leipzig, 1915. 57 pages, 29 text figures and 2 plates. M 2.80.

The cause of hoof and mouth disease of cattle has been variously assigned, usually on *a priori* grounds, to bacteria, protozoa, and to ultra-microscopic organisms, but no one except the author of the present work has succeeded in satisfying the necessary postulates for the determination of a disease-causing parasite. Dr. Stauffacher of Frauenfeld, Switzerland, has found definite bodies in the blood and diseased tissues of every animal with the disease examined; he has grown these bodies in the condensation water of blood agar culture media and has observed many developmental stages; he has inoculated normal cattle with the organisms from the artificial cultures and produced the disease in such previously unaffected animals, and he has recovered the organisms from the diseased tissues of the inoculated animal.

Students of hoof and mouth disease have been bull-dozed by authority into the view that the cause must be ultra-microscopic, first, because no trace of organisms can be found in diseased tissues treated and stained by the ordinary technical methods, and second, because the virus passes through the ordinary filters. When no less an authority than Löffler implies that it is a waste of time to look for the organisms of hoof and mouth disease with a microscope, it is to be expected that only those investigators who have authority-proof minds will undertake the task. Such men do not forget the history of *Treponema pallidum*. Nor is the fact that the virus of hoof and mouth disease will pass through a filter, necessary evidence of ultra-microscopic size; trypanosomes, to say nothing of spirochaetes, pass through ordinary bacteria filters.

Stauffacher seems to have such an authority-proof mind; he argued that the organisms must be in the infected tissues, and that the fact of their not taking the ordinary stains is no guarantee that they may not stain with altered methods. So after vainly trying to stain sections with haematoxylin, fuchsin-methylen-blue, and other anilin combinations, finding no characteristic chromatin reaction in the infected cells, either in the nuclei or cytoplasm, he substituted acid fuchsin for the ordinary fuchsin and found, as Zschokke had found before, that infected tissues take up more acid fuchsin than do normal tissues. Even with this modification however, he was unable to get any trace of basichromatin material either in the nuclei or in the cell bodies. Finally, after "months of study of thousands of sections" he discovered the important secret of treatment, after which the nuclei again took up the

characteristic methylen-blue stain, while thousands of minute bodies in and around the cells, and not seen before, were revealed. The method used was very simple: the sections were first stained for from two to six hours in dilute aqueous acid fuchsin (0.2 per cent), rinsed in distilled water and then stained for from six to ten hours in Ehrlich's fuchsin-methylen-blue mixture, then rinsed thoroughly in distilled water after which they were left in absolute alcohol until no more color came out, and were cleared in xylol and mounted in balsam.

The important part of Stauffacher's work centers in the cell inclusions and the similar bodies found in the lymph spaces and in the blood of infected animals. These are minute polymorphic structures with an average length of one micron (1 μ), and either spherical, ellipsoidal, crescentic, chain-form, comma-form, or ring-form in shape. They were found in all of the infected animals (26) examined, and were never found in similar tissues of normal, healthy animals. The same bodies were found in the freshly-drawn blood, both free in the plasma and within the red blood corpuscles of infected animals, a fact which practically excludes the possibility that they are products of nuclear and cellular degeneration brought about by the disease.

Blood was drawn from the jugular vein of an infected animal under sterile conditions, and a few drops were added to sterile tubes of blood agar prepared according to Nicolle's formula for growing *Leishmania*. Vesicles on the tongues of infected cows were flooded with sterilized distilled water which was then withdrawn and placed in similar agar tubes, one cubic centimeter to each tube. On the fourth day afterwards, the tubes containing the vesicular lymph had a decidedly milky appearance. A drop of this, examined under the microscope, showed myriads of actively moving organisms. The tubes with the venous blood showed the same picture somewhat later. Two distinct types of organisms were observed; one, shorter and thicker, had the characteristic appearance of a flagellated protozoon, with a lancet-formed body which becomes sharply attenuated and drawn out into a long flagellum. The average length of these individuals was 45 μ of which the body comprised from 20 to 25 μ with a diameter of about 3 μ. The second type was much longer and more thread-like, with maximum dimensions of 120 μ by 1 μ. Granules within the bodies of these two types were regarded as blepharoplast, nucleus and chromidia; the bodies themselves were not metabolic, nor was there any evidence of mouth or vacuoles. Reproduction by longitudinal division was common to all types. In addition to reproduction by division, another method analogous to spore-formation was described; in this the body becomes thickly strewn with chromidia, each of which becomes the minute nucleus of an excessively small spherical structure, similar to some of the intra-cellular stages, and which might well be able to pass a filter.

Finally Stauffacher inoculated two normal cows with the uncontaminated agar culture material. The first experiment was interrupted by the mobilization of the Swiss army in August, 1914, and we are not told what became of the animal. The second experiment was successful,

the animal developing symptoms of the disease on the fourth day after subcutaneous injection of culture material. From this animal the contents of a fresh vesicle was p aced in an agar tube, and in two days the fluid was swarming with the same kinds of organisms that had been introduced from the earlier culture tube.

From the standpoint of protozoology these results are entirely consistent with the established facts of the life histories of other cell-invading flagellated parasites. It is regrettable that the technique was such that no definite conclusions can be drawn in regard to the finer structures of the parasite. All of the tissues and smears were fixed in 70 per cent alcohol which is far from satisfactory for the interpretation of delicate structural details. Observations on the living organisms leave no doubt of the flagellum, but we do doubt a statement to the effect that this flagellum "is not a flagellum in the usual sense of the word, but rather a flagellum-like appendage to the cell." A better fixation and more careful staining would probably reveal the nucleus and blepharoplast and the insertion of the flagellum base in or near the blepharoplast. The polymorphic structure of the organisms is not an uncommon feature of allied forms of protozoa; short and stumpy types mingled with long thread-like forms are characteristic of cultural stages of *Crithidia*, *Leptomonas*, *Trypanosoma* and *Leishmania*, and similar differences in form are met with in the normal hosts.

The intra-cellular bodies with their varied forms, which, however, are reducible to one general type, are strikingly suggestive of *Leishmania donovani* of kala azar. But here again, the finer structures must remain unknown until a better method of fixation is employed.

On the whole, we are inclined to accept Stauffacher's interpretation of the organism which he names *Aphthomonas infestans*, as belonging to the group of simple flagellated protozoa (Monadida) and closely related to the genus *Leishmania*. If this work is confirmed on material fixed with better methods, we should naturally expect the next step to be the discovery of the transmitting agent in some form of tick or biting fly.

GARY N. CALKINS.

Columbia University,
March, 1916.

ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

ANIMAL PATHOLOGY

The Bacillus enteritidis as the Cause of Infectious Diarrhea in Calves. K. F. MEYER, J. TRAUM, C. L. ROADHOUSE. (Jour. Am. Vet. Med. Assoc., 1916, **49**, 17–35.)

In the course of an experiment on feeding calves at the Agricultural Experiment Station of the University of California, infectious diarrhea or scours occurred in severe form. The etiological agent was determined to be of the *paracolon* type by all the identity reactions and serological tests; and was regarded as identical with *B. enteritidis* (Gärtner). Bacteriological findings were confirmed by feeding experiments with two calves, of which one succumbed and one recovered.—A. R. W.

A Filterable Organism Isolated from the Tissues of Cholera Hogs. D. J. HEALY and E. J. GOTT. (Jour. Infect. Diseases, 1916, **18**, 124–128, 1 pl.)

In the course of a previous attempt to isolate a filterable virus from the mesenteric glands of virus hogs, the technique involved the filtration of the glands immediately after grinding. In the present investigation the gland tissue after grinding was suspended in 1 per cent glucose neutral beef broth at 4°C. for five days, then put through a tested Chamberland-Pasteur "F." The filtrate was divided between two flasks, one of which was placed in a Novy jar, the other sealed, and both incubated at 4°C. While the anaerobic preparation showed no growth after thirteen days, the other flask showed a distinct growth after four days. "This growth appeared as a fine sediment in the bottom of the flask." Upon agitation it "ascended through the fluid in the shape of a small cloud. . . ." This "filterable organism" grew best at 37°C. but also at 20°C. and at 4°C. Hanging drop preparations revealed clumps of a non-motile organism surrounded by a gelatinous material. Satisfactory stained preparations were obtained by the Giemsa method. In such preparations the organism appeared as a coccus or a small bacillus 0.2 to 0.3μ in diameter. Subcultures were not obtained. In tests with immune serum complement fixation was obtained with the culture fluid in which the organism had been grown.—P. B. H.

A Report Upon an Outbreak of Fowl Typhoid. WALTER J. TAYLOR. (Jour. Am. Vet. Med. Assoc., 1916, **49**, 35–47.)

The writer encountered in California an outbreak of the disease first described by Moore in 1895, as infectious leukemia, and by Daw-

son in 1898, and studied under the name, fowl typhoid, by Curtice in 1902.

His observations led Taylor to the following conclusions:

1. Fowl typhoid is a specific disease of fowls caused by *Bacterium sanguinarium* occurring sporadically and causing heavy losses among affected flocks; which unless properly investigated may easily be mistaken for fowl cholera because of its high mortality.

2. The specific morbid conditions consist of an enlarged liver containing necrotic areas, an enlarged spleen and a general anemic condition of the serous and mucous membranes together with a marked increase in leucocytes and a corresponding decrease of the red cell content of the blood.

3. The increase in leucocytes seems to be confined to the polymorphonuclear variety.

4. Fat, well conditioned, adult fowls are more susceptible than young, nearly mature growing birds.

5. Birds may contract the disease by the ingestion of pure cultures of *Bacterium sanguinarium*.

6. Birds fed upon the offal of other birds dead of this disease show a mild non-fatal form of the disease tending to recovery.

7. There is evidence that recovery from this mild form produces more or less of an immunity. Further investigation upon this point is needed.

8. The power of some of the red corpuscles of the affected fowls to take the violet stain, when the blood is diluted in Toisson's fluid is especially noticeable in this disease.

9. While the lesions produced in fowls which are infected with *Bacterium sanguinarium* resemble in many respects those produced by *Bacterium pullorum*, and although there is a still closer resemblance in the biological characters of the two organisms, there is enough difference to warrant the conclusion that they are distinctly different diseases.

A. R. W.

The Value of Virulent Salt Solution in the Production of Anti-Hog-Cholera Serum by the Intravenous Method. ROBERT GRAHAM and L. R. HIMMELBERGER. (Jour. Infect. Diseases, 1916, **18**, 118–123.)

Craig and Robbins have each shown that salt solution rendered virulent by remaining for some time in the peritoneal cavity of virus pigs can be used advantageously by subcutaneous inoculation to produce potent antisera in immune hogs. The present authors attempted to apply this method modified by the use of intravenous inoculation. A virulent salt solution was obtained by injecting into the intraperitoneal cavity 25 cc. of a 0.9 per cent solution per pound weight of hog. The salt solution, recovered at time of killing five hours later, represented 40 to 70 per cent of the original volume. The volume of blood obtained at the same time was found to have increased on the average by 10 to 20 per cent. Salt virus and blood virus, mixed in the proportions of 1:1 or 3:1, and inoculated either subcutaneously or

intravenously at the rate of 7 cc. per pound of body weight into immunes gave satisfactory results in the production of potent antisera when these sera were tested on susceptible shoats infected with 2 cc. of virus. The conclusions are reached that an immune serum may be produced and used more economically when mixed salt virus and blood virus are employed and injected by the intravenous method, since the total volume of available virus solution is raised by this procedure from 75–80 per cent.—P. B. H.

Experiments to Determine the Relative Value of Trikresol and Carbolic Acid in the Preservation of Hog Cholera Serum. JOHN REICHEL. (Mulford's Vet. Bul., 1916, **7**, 61–64.)

Carbolic acid is generally used as the preservative of hog cholera serum, even though comparatively little is known of its value as a germicide in such a product as hog cholera serum in the form of defibrinated blood as originally prepared by Dorset, McBryde and Niles. That carbolic acid itself has little or no effect on the potency of the product is conclusively established.

Whether trikresol is equally harmless and as effective as a preservative remains to be shown.

Hog cholera serum as generally prepared is not sterile. The blood as drawn from the serum-producing animal is invariably contaminated and subsequent handling in defibrination allows for additional contamination, up to the time the preservative is added, varying in degree with the care exercised in its preparation. Even though it is possible to obtain sterile blood from a serum-producing hog, this can only be said of experimental trials and in producing hog cholera serum defibrinated blood, in a practical way, the question of sterility must be entirely sacrificed.

Carbolic acid will not sterilize this contaminated product and the question naturally arises "does it hold the organisms in check whether present in large or small numbers?"

The writer observes that:

1. Carbolic acid must be used in less than 0.75 per cent to avoid changes in the physical appearance of hog cholera serum defibrinated blood.
2. Trikresol must be used in less than 0.6 per cent for the same reason.
3. Carbolic acid added in amounts up to 0.75 per cent to lightly or heavily contaminated defibrinated blood first caused a decrease in the number of bacteria followed by an increase exceeding the first bacterial count.
4. Trikresol with a carbolic acid coefficient of 2.87 added in amounts up to 0.6 per cent was also followed by a decrease, then an increase practically equal to that which occurred in the carbolized samples.
5. All of the controls, without any preservation, showed an increase in the bacterial count from the time the samples were first set aside along with those to which a preservative had been added. This

increase was followed by a noticeable decrease, and the last count was on an average lower in the control samples than those treated. That putrefactive changes took place was appreciated by the odor and liquefied appearance of the product.

6. No odor or putrefactive changes were observed in the carbolized and trikesolized samples. Both preservatives served well in this respect, but this alone must not be accepted as proof of the value of either preservative for defibrinated blood.

7. From the limited number of examinations made in these experiments as to the types of bacteria which survive and then flourish in the carbolized and trikresolized samples, it can be said that the types were not limited to the spore-forming bacteria alone, as organisms of the colon type, staphylococci and streptococci were found as long as the samples were kept.

8. Contaminated hog cholera serum defibrinated blood cannot be sterilized by the addition of carbolic acid and trikresol in practical amounts, and the numbers of bacteria are not kept in check by the preservative.

9. No evidence is brought forth here to show that carbolic acid or trikresol would not serve well as preservatives when added to a sterile product.

10. Hog cholera serum must be prepared in a sterile manner or sterilized by one means or another to enable carbolic acid or trikresol to serve as a satisfactory preservative.

11. The physical nature of hog cholera serum defibrinated blood, probably has much to do with the limitations of carbolic acid and trikresol as preservatives, and it is highly probable that both would prove more effective if the insoluble, inert material, fibrin cellular débris, etc., were eliminated from hog cholera serum. These inert materials undoubtedly exert a large influence in the complications following the use of the product, and for this reason alone should not be allowed to remain in hog cholera serum on the market.—A. R. W.

BACTERIOLOGY OF AIR AND DUST

Recovery of Streptococcus viridans from New York Street Dust. W. C. Thro. (New York Med. Jour., 1916, **103**, 444–445.)

Cultures made from New York street dust, collected at the level of the second floor revealed the presence of *Bacillus fluorescens* once; a member of the colon group, probably paracolon, once; chromogenic Gram positive cocci several times; and *Streptococcus viridans* seven times. The strains of *Streptococcus viridans* were tested for their fermentative properties and their pathogenicity for mice and rats.

M. W. C.

BACTERIOLOGY OF FOODS

Food Poisoning by the Bacillus Paratyphosus B. HARRY S. BERNSTEIN and EZRA S. FISH. (Journ. A. M. A., 1916, **66**, 167.)

Report is made of an epidemic of food poisoning at Westerly, R. I. Sixty persons were made seriously ill, four of whom died. Symptoms of gastric disorder occurred within 4½ to 19 hours after eating pie obtained from a local restaurant.

In an analysis of the ingredients of the pies an organism was isolated possessing the morphologic, serologic and cultural characteristics of *Bacillus paratyphosus B.*—G. H. S.

Indol in Cheese. V. E. NELSON. (J. Biol. Chem., 1916, **24**, 533.)

N. determined presence of putrefactive products, indol, skatol and phenol in different cheeses. Indol present in limburger and camembert, phenol only in former. Skatol absent in both. Cheddar, swiss and roquefort are free from these substances. Lactic and bulgarian bacilli and a liquefying coccus were grown in a medium containing tryptophan, 4 per cent lactose and salts. First two failed to produce indol; last produced small amounts. N. believes that medium was probably unfavorable for growth of former. He apparently disregards the sparing effect of lactose.—I. J. K.

BACTERIOLOGY OF SOILS

Relation of Green Manures to the Failure of Certain Seedlings. E. B. FRED. (J. Agr. Res., 1916, **5**, 1161–1176.)

It has been observed that germination of seeds is poor on soil to which green manures have been recently added. The writer reports the result of an investigation to show whether this may not be due to micro-organisms (bacteria or fungi) that develop in large numbers in decomposing green manures. The indications point to fungi as the harmful agents. Some fungi have been isolated from decomposing green clover that are very destructive to seedlings. Oily seeds are easily damaged by the fungi, but starchy seeds are very resistant. Damage to seeds by green manures is generally confined to the first two weeks after their addition to the soil. Small applications of calcium carbonate seem to increase the injury. In all cases where the germination is slow, a high percentage of the seedlings prove to be diseased.—H. J. C.

Relation of Carbon Bisulphid to Soil Organisms and Plant Growth. E. B. FRED. (J. Agr. Res., 1916, **6**, 1–19.)

It has been shown in the past that if soil is treated with carbon bisulphid there is an initial decrease in the number of micro-organisms which is followed by a large increase in their numbers and in the amount of nitrate and ammonia produced, as well as by increased plant growth. The writer confirms these conclusions.

One theory commonly held to explain these results is that carbon bisulphid kills certain of the micro-organisms, thus allowing other kinds to increase to abnormal numbers and to supply the plants with an unusual amount of available nutrient matter. Another theory, held by A. Koch, is that the carbon bisulphid in the small quantities used is a direct stimulant to bacteria and to higher plants.

The writer's data tend to support Koch's theory. Carbon bisulphid does not act alike in all soils or toward all crops. There is an increased growth of plants in sand culture (pure silica sand with nutrient solution added) as well as in soil. Of all the crops investigated, mustard (which contains sulphur) receives the most striking benefit from the treatment (except in acid soils). Next to mustard comes rape, then red clover, then buckwheat, then oats, while corn is scarcely benefited if at all.—H. J. C.

BACTERIOLOGY OF THE MOUTH

Deep Seated Alveolar Infections. M. L. Rhein. (Surg., Gynecol., and Obstet., 1916, **22**, 33–37.)

One case of arthritis is mentioned. *Streptococcus viridans* was isolated from the pulp of an apparently sound tooth. Patient made good recovery.—C. P. B.

The Dental Aspect of the Relation of Endamoeba to Pyorrhea Alveolaris. W. A. Price, D.D.S., M.S. (Surg., Gynecol., and Obstet., 1916, **22**, 37–43.)

The author's subject was really Cinematographic Film studies showing the movements of mouth organisms, including endamoeba. This method should lend itself to studies of most bacterial forms.

C. P. B.

On the Cultivation of Entameba buccalis. Wm. B. Wherry and Wade W. Oliver. (Lancet Clinic, 1916, **115**, 295.)

Wherry and Oliver found that the *Entameba buccalis* grew best on "Martin's pleuritic" medium, made up with basic sodium phosphate, and pleuritic fluid in the proportion of about two of the fluid to three of the agar. The tubes were slanted and allowed to remain in the ice box, so that the water of syneresis could collect. The *Entameba* and bacteria from the margin of the tooth were introduced into the water of syneresis and incubated at 35° to 37°C. There was a profuse growth at the end of 48 hours; staining with Mallory's ferric chloride-hematoxylin, showed characteristic nuclear structure. The authors hope to be able to throw some light on the life history of this organism and to make tests of its pathogenicity.—O. B.

The Relation of Amoebiasis to Pyorrhea Alveolaris. A. H. Sanford, M.D. and Gordon B. New, M.D. (Surg., Gynecol. and Obstet., 1916, **22**, 27–33.)

The authors studied 327 patients, dividing them into 5 groups. Material from pyorrheal pockets, also from sto ls was examined. Of

73 patients with *Entameba histolytica* present in the stools only 31 also had *Entameba buccalis* in the mouth while in 254 cases in which parasites were absent from the stools, 150 had *Entameba buccalis* present.

Dogs were inoculated about the teeth with pus containing amoebae from pyorrheal pockets. In subsequent examination no signs of pyorrhea were present and amoebae could not be found.

Kittens given intracaecal inoculations of *Amoeba histolytica* developed typical dysentery while those inoculated with *Amoeba buccalis* showed no signs of dysentery.—C. P. B.

The Dental Path: Its Importance as an Avenue to Infection. T. B. Hartzell, M. D. and A. T. Henrici, M.D. (Surg., Gynecol., and Obstet., 1916, **22**, 18–27.)

In a series of acute dental abscesses staphylococci were the active organisms, in 250 cases of chronic abscess *Streptococcus viridans* was the predominating organism.

Heart lesions were produced in rabbits, injected with cultures of *Streptococcus viridans* and other streptococci obtained from pyorrheal pockets and saliva, while some strains seemed to have a predilection for the kidneys.

In one case death was due to the fusiform bacillus, the organism being isolated from the blood. Another died of pneumonia, the primary infection with the pneumococcus occurring in a bicuspid tooth.

Comment. Many authors now classify cocci producing greenish color on blood agar, not pneumococci, as *Streptococcus viridans.* It would seem desirable that the authors should determine whether the organism is the classic *Streptococcus viridans,* that is a pin head grayish rough irregular colony, which gradually becomes brownish producing a greenish color, in the blood agar surrounding the colony and grows in very long chains in bouillon, the elements of which are diplococci; or simply belongs to the group which produces the green color when grown on blood agar.—C. P. B.

BACTERIOLOGY OF WATER AND SEWAGE

The Electrical Treatment of Water. T. A. Starkey. (American Medicine, 1916, **11**, 181.)

S. passed currents of electricity through water containing *B. coli* and *B. prodigiosus.* He found that the current as such had very little effect on the germs. He used both direct and alternating currents and varied the amperage from 0.1 to 2.0 and the voltage from 90 to 40,000.

He found that the gases produced by the electrolysis did have some germicidal power. In one case the count was reduced from 34,000 to 2,000. In no case was the water made entirely sterile. S. does not state whether there was any salt in the water from which hypochlorite might have been made.

In another series he tried to test the sterilizing value of any metallic

salts that separated from the plates (electrodes) and obtained complete sterility in a few cases. He does not, however, state what material composed the plates or what salts passed out into the water. The report is so incomplete as to be of little value.—E. C. L. M.

CLASSIFICATION OF BACTERIA

Grouping of Meningococcus Strains by Means of Complement Fixation. MIRIAM P. OLMSTEAD. (Proc. N. Y. Pathol. Soc., 1915, **15**, 136–143.)

Forty strains of meningococci were used but only twenty-nine were completely tested by complement fixation. Of these, fourteen fell into one group, eight into another,two cross-fixed with each other only, two failed to fix with any other strain and three acted irregularly. All strains could be clearly distinguished from the gonococcus. Culturally they were all alike. The para-meningococci are regarded as constituting a special strain among meningococci, not, however, a wholly homogeneous one.—W. J. M.

DISINFECTION

The Standardization of Disinfectants. J. T. AINSLIE WALKER. (New York Med. Jour., 1916, **103**, 500–505.)

A critical comparison of the Hygienic Laboratory and Rideal-Walker tests, leading to the conclusion that the Rideal-Walker test is superior to the Hygienic Laboratory method in every one of the points discussed. M. W. C.

Soap. G. K. DICKINSON. (Medical Record, 1916, **89**, 556–558.)

Work done up to the present time upon the antiseptic and bactericidal action of soap has not been sufficiently uniform in conditions or methods to draw from it hard and fast conclusions.

In general, it may be said that all soaps possess some disinfectant power by virtue of the alkaline reaction alone. All bacteria exposed to soap solutions are not, however, killed in the same time, and a considerable interval is necessary before any practical disinfection can occur. Most so-called disinfectant soaps have no value beyond that of ordinary soaps. A combination of soap and biniodide of mercury is a useful disinfectant, but it does not produce complete sterility M. W. C.

IMMUNOLOGY

Therapy as Related to the Immunology of Tuberculosis. E. R. BALDWIN. (New York Med. Jour., 1916, **103**, 532–534.)

A discussion of the relation of immunology to the methods of treatment now used in tuberculosis.—M. W. C.

Vaccines of Favus and Ringworm. C. H. LAVINDER. (Journ. A. M. A. 1916, **66**, 945–946.)

A method is given for the preparation of vaccines from the fungi which cause the above infections.—G. H. S.

Treatment of Cases of Epidemic Meningitis. J. B. NEAL. (Journ. A. M. A., 1916, **66**, 862–864.)

The author places emphasis upon the value of continued injections of antimeningitis serum even though the patient shows improvement.

Autogenous vaccines have been employed in cases which tended to become chronic.—G. H. S.

The Effect of Moderately High Atmospheric Temperatures upon the Formation of Hemolysins. C.-E. A. WINSLOW, JAMES ALEXANDER MILLER, and W. C. NOBLE. (Proc. Soc. Exp. Biol. and Med., 1916, **13**, 93–98.)

Rabbits kept at a temperature of 29° to 32°C. were compared with control rabbits kept at 18° to 21°C. by injecting them with washed sheep erythrocytes and subsequently testing the specific hemolytic activity of the serum. Hemolysin formation was relatively delayed in the animals kept at the higher temperature, but at the end of four weeks the titre was as high in these as in the controls. Considerable individual variation within the groups was observed.—W. J. M.

An Allergic Skin Reaction to Diphtheria Bacilli. J. A. KOLMER. Proc. Soc. Exp. Biol. and Med., 1916, **13**, 89–91.

Diphtherin was prepared by suspending washed diphtheria bacilli in salt solution and sterilizing the suspension at 60°C. for an hour. Each cubic centimeter contained approximately two billion bacilli. An intracutaneous injection of 0.1 cc. of diphtherin was used for the test, and the usual Schick test was also made at the same time. The two tests agreed in 63 per cent of the patients tested. The diphtherin test is regarded as an index of bacteriolytic immunity whereas the Schick test is an index of antitoxic immunity.—W. J. M.

The Influence of Typhoid Bacilli on the Antibodies of Normal and Immune Rabbits. C. G. BULL, Journ. Exp. Med., 1916, **23**, 419–429.

The subcutaneous, intraperitoneal, or intravenous inoculation of cultures of typhoid bacilli did not cause, as far as could be determined, a decrease in the antibody content of the blood serum of the rabbit. On the other hand, the intravenous inoculation of typhoid bacilli causes a rapid mobilization of antibodies, thus increasing their concentration in the blood, to be followed somewhat later, by the production of so-called acquired antibodies. No such condition as the negative phase of Wright was discovered, although it was especially looked for in the experiments.—G. B. W.

A Method for the Rapid Preparation of Anti-Meningitis Serum. H. L. Amoss and Martha Wollstein. (Jour. Exp. Med., 1916, **23**, 403–417.)

The method described by the authors consists of three successive intravenous inoculations of many strains of living meningococci and parameningococci repeated at stated intervals. Anaphylactic dangers are obviated by preliminary desensitizing injections and the doses are adjusted according to the febrile reaction. The great advantage of the method is that a polyvalent serum of high titer can be produced in 8 to 12 weeks instead of in the 10 months required by the subcutaneous method. This investigation offers a promising suggestion for the production of other immune sera.—G. B. W.

Variations in the Strength of Positive Wassermann Reactions in Cases of Untreated Syphilis. D. A. Haller. (Journ. A. M. A., 1916, **66**, 882–884.)

From an examination of over 6000 Wassermann reactions in which but one antigen was used it appears that amboceptor is the only constituent of the hemolytic system which is a constant. All other factors may vary and will account for the difference in daily determinations of the fixing unit of a positive serum.

The titer of sera from cases of untreated syphilis remains the same from day to day or from month to month.

The administration of mercury may quickly change a positive to a negative reaction, and upon stopping the treatment the reaction may as quickly become positive again.—G. H. S.

Continuous Transfusion; The Production of Immunity. An Experimental Study. A. Kahn. (Medical Record, 1916, **89**, 553–556.)

Dogs were infected by opening the peritoneal cavity and inserting a small quantity of dust, gauze saturated with pus, or pure pus. After an interval, varying from 1 to 5 days, the donor and the infected dog were prepared for transfusion and a continuous flow of blood from one animal to the other was allowed to occur from ½ to 3 hours.

In dogs that were not transfused following infection, death occurred in 24 to 48 hours; in transfused dogs death was deferred from 3 to 4 days, or absolute recovery took place.

Transfusion raises the vital resistance. Whether or not immunity is produced is not known.—M. W. C.

Possible Reasons for Lack of Protection after Antityphoid Vaccination. Henry J. Nichols. (The Military Surgeon, 1916, **38**, 263–268.)

Summary of article as given by author is:

1. False failures in immunization may be due in some cases to the difficulties of exact clinical diagnosis.

2. The uncertain duration of immunity following vaccination may account for some true failures. At present in the Army one revaccina-

tion of three doses is compulsory four years after the first course of three doses.

3. The kind of vaccine used may account for some failures.

a. Whole killed vaccine.

(1.) The strain of bacillus used may not be suitable.

(2.) The method of preparation may be faulty.

(3.) The vaccine may be too old.

b. Sensitized vaccine.

(1.) Sensitization may diminish the immunizing properties of the vaccine.

(2.) Discarding the supernatant fluid may lessen the immunizing power of the vaccine.

4. The conditions of a soldier's life do not protect him from exposure to typhoid fever in his vicinity.

5. The Army vaccine is probably superior to some of the vaccines available for the general population.

The writer comes to the conclusion that soldiers are better protected than those who become infected after vaccination in civil life, and that this protection must be due either to better vaccination in point of numbers of doses and intervals of revaccination, or to a better vaccine. Of the two factors the vaccine is probably the most important.

E. B. V.

Methods of Using Diphtheria Toxin in the Schick Test and of Controlling the Reaction. ABRAHAM ZINGHER. (American Journal of Diseases of Children, 1916, **4**, 269–277.)

The Schick reaction consists in the intracutaneous injection of one-fiftieth M.L.D. of well ripened diphtheria toxin and indicates the absence or presence of a protecting amount of antitoxin in the blood according to whether there is or is not produced a local inflammatory reaction. In some individuals there occurs a so-called pseudo-reaction which probably bears no relation to the free toxin but is the result of an anaphylactic reaction with the proteins of the diphtheria bacilli. The true and the false reactions can usually be distinguished by their appearance and time of occurrence but as a further control a superheated (75°C.) toxin or one which has been over neutralized by the addition of two units of antitoxin to each L plus dose of toxin may be injected into the opposite arm.

The author also emphasizes the importance of careful technique in giving the injection so that it is definitely intracutaneous. The value of the test now seems well established and especially prepared vials containing undiluted toxin and with directions concerning its dilution are prepared by the New York City Board of Health and also by commercial laboratories.

Of a thousand children admitted to the Willard Parker Hospital, who gave a negative Schick reaction and who were more or less exposed to diphtheria not a single one developed the disease.

Tests on 2700 normal children in orphan asylums between the ages

of 2 and 16 years show that from 17 to 32 per cent give a positive reaction and are therefore probably susceptible to the disease.

R. M. T.

Preliminary Notes on Skin Reactions Excited by Various Bacterial Proteins in Certain Vasomotor Disturbances of the Upper Air Passages. J. L. GOODALE. (Boston Med. and Surg. Jour., **174**, 223–226.)

G. finds that many patients who suffer from perennial vasomotor disturbances of the nasal mucous membrane give a positive anaphylactic skin reaction to extracts of certain bacteria that are commonly found in vasomotor rhinitis. Among these are *Staphylococcus albus, aureus* and *citreus, Micrococcus tetragenus* and an unidentified bacillus somewhat like Friedlaender's bacillus.—E. C. L. M.

Pollen Therapy in Pollinosis. S. OPPENHEIMER and M. J. GOTTLIEB. (Medical Record, 1916, **89**, 505–508.)

Hay fever, or pollinosis, is caused in persons having a predisposition to anaphylactic diseases, by irritation of any denuded surface of the body by proteins of pollen. One or more of a large variety of pollens may be responsible for an attack of pollinosis in a susceptible individual.

The method most frequently employed for determining which pollen or pollens are operative in a given case is the skin scarification or cutaneous method. Complement fixation tests may also be made both as an aid in diagnosis and as an indicator of immunity.

Infections, caused by streptococci, pneumococci, etc., are often complicating factors in pollinosis. In such cases an autogenous vaccine should be administered in conjunction with the specific pollen antigen.

Treatment of pollinosis proper may consist either of active immunization with pollen extract or passive immunization with the blood serum of animals that have been actively immunized with pollen extract.

The results obtained with treatment with pollen extract, in cases of spring pollinosis show 50 per cent of seasonal cures for 1913–1914, while of 32 cases treated in 1915 before the time of attack, only two had symptoms.

Of 62 cases treated for fall pollinosis 52 began treatment early enough to acquire an active immunity before the usual time of attack. Of these, 15 were free from symptoms, 25 were markedly improved, and 12 were in no way affected by the treatment. Of the 10 cases that did not begin treatment until after the onset of the attack, 4 were favorably influenced by one or two injections.

The authors caution against using "hay fever" vaccines which contain a mixture of a large number of pollen extracts, as patients should receive extracts of only those pollens which have been shown by diagnostic means to be operative. Care should be taken in the dosage of pollen extracts, as in large doses they are extremely dangerous.

M. W. C.

Complement Fixation in Intestinal Parasitism of Dogs. JOHN A. KOLMER, MARY E. TRIST and GEORGE D. HEIST. (Jour. Infect. Diseases, 1916, **18**, 88–105.)

The aim of the investigation was to ascertain by complement fixation tests whether the absorption of foreign substances and consequent production of specific antibodies occurred in dogs infected with intestinal parasites. For antigens were used salt solution and alcoholic extracts of various species of *Tenia*, *Dipylidium*, *Ascaris* and *Strongylus*. The antisheep hemolytic system was employed. Each one of 172 dog sera was tested with all of the antigens. Serum tests and feces examinations together were made in 110 cases. The results of feces examinations showed infections as follows: *Ascaris* (23 per cent), *Ascaris* and *Trichocephalis* (20 per cent), *Tenia* (6 per cent), *Dipylidium* (3.6 per cent), no infection (26 per cent). These results did not conform with the serum examinations since (1) dogs showing the ova of certain parasites failed to react with the corresponding antigen, and (2) positive reactions were frequently obtained with antigens of types whose eggs were not found in the feces. The analysis of the data, however, leads the authors to conclude that the production of antibodies may occur after infestation with the common intestinal parasites. Such antibodies were in special evidence in tapeworm infestations, less so in round worm and only slightly in whip worm infestations. The reactions as a whole are stated to have suggested a biologic relation between the tapeworms *Tenia serrata* and *Dipylidium caninum*, and between *Ascaris canis* and *Strongylus gigas*. The authors state that complement fixation tests may be of value in the diagnosis of intestinal parasitism of man.—P. I. H.

Studies in Non-Specific Complement Fixation: I. Non-Specific Complement Fixation by Normal Rabbit Serum. JOHN A. KOLMER and MARY E. TRIST. (Jour. Infect. Diseases, 1916, **18**, 20–26.)

The authors direct attention to the fact that fresh active sera from normal rabbits, in doses of 0.1 c.c. show non-specific fixation with lipoidal extracts in 5 to 15 per cent of sera tested. When the same sera were inactivated by heating fixation occurred in 38 to 49 per cent of sera. In the case of both active and inactivated sera the percentage of positive reactions increased in the following order when the substances named were used as antigens: (1) alcoholic extract of heart muscle reinforced with cholesterin, (2) alcoholic extract of syphilitic liver, (3) extract of acetone insoluble lipoids. With bacterial antigens (staphylococci, colon, typhoid) ixation occurred in some degree in 31 to 42 per cent of cases, with active sera, and in 51 to 62 per cent when inactivated sera were used. The rabbits tested were conservative in their reactions, 80 per cent being persistently positive or persistently negative in successive examinations. The authors conclude by recommending that "when rabbits are to be employed for experimental studies with a view to using their sera for complement-fixation tests, their sera should be tested one or more times before inoculation preferably

with the particular antigen to be used, and only those selected that react negatively."—P. B. H.

Studies in Non-Specific Complement Fixation: II. Non-Specific Complement Fixation by Normal Dog Serum. JOHN A. KOLMER, MARY E. TRIST and GEORGE D. HEIST. (Jour. Infect. Diseases, 1916, **18**, 27–31.)

The study was undertaken to ascertain whether normal dog serum would fix or absorb complement with lipoidal and bacterial antigens as had been found to be the case with normal rabbit serum. The technique was that of the Wassermann reaction. It was found that the dog sera tested, whether active or inactivated, are capable of absorbing complement in a large percentage of cases, the greater number of positive reactions appearing in the case of bacterial antigens. When lipoidal antigens were used the order of positive reactions varied exactly as in the case of rabbit serum (*vide supra*). The best reactions with active dog serum were obtained when 0.05 cc. was used. Heating the sera at 55°C. for 30 minutes greatly increased the power for fixation for both groups of antigens, while by heating at a higher temperature the power was lessened. The authors conclude that in complement fixation tests with dog serum, "it would appear advisable to use the serum in a perfectly fresh and active condition in doses of 0.01 to 0.2 cc., after heating the serum at 62°C. instead of 55°C. for half an hour, since this removes, or greatly diminishes the tendency toward non-specific fixation of the complement."—P. B. H.

Studies in Non-Specific Complement Fixation: III. The Influence of Splenectomy and Anesthetics on the Non-Specific Complement Fixation Sometimes Shown by Normal Rabbit and Dog Sera. John A. KOLMER and RICHARD M. PEARCE. (Jour. Infect. Diseases, 1916, **18**, 32–45.)

The aim of the investigation was to gain some understanding of the part played by the spleen in hemolysis, and in the increased resistance of erythrocytes after splenectomy. Pre-operative and post-operative sera were tested in both active and inactivated condition in doses of 0.1 cc. against three lipoidal extracts (*vide supra*) and two bacterial antigens (Staphylococci and *B. coli*). Ether, chloroform and nitrous oxid were employed as anesthetics. The results of the experiment showed that anesthetics, as employed, weaken or remove temporarily the power of normal rabbit and dog sera of fixing or absorbing the complement with lipoidal and bacterial antigens in a non-specific manner. "This alteration usually is not apparent at once after the administration of the anesthetic but is found after one to three days; later the serum returns to its former power of causing this non-specific fixation." Ether-administration was not found to reverse the reaction of negatively-reacting sera. Nitrous oxid oxygen had no appreciable influence on the serum reaction of normal rabbits. "Splenectomy alone probably has no influence upon the property in normal rabbit and

dog sera of fixing or absorbing complement with various non-specific lipoidal and bacterial antigens, the effect being in larger doses attributable to the anesthetic; the changes observed in dogs following splenectomy under ether were somewhat more profound than those in rabbits."

P. B. H.

Studies in Non-Specific Complement Fixation: IV. The Relation of Serum Lipoids and Proteins to Non-Specific Complement Fixation with Normal Rabbit and Dog Sera. JOHN A. KOLMER. (Jour. Infect. Diseases, 1916, **18**, 46–63.)

The aim of the present study was to determine the relation of serum lipoids to the process of non-specific complement fixation (1) by extractions of serum with lipoid solvents (ether, chloroform, etc.), and (2) by feeding and immunization experiments with various lipoids. The method employed antilytic and Wassermann tests with rabbit and dog sera, both active and inactivated (56°C. for one-half hour), before and after extraction. It was found that both serum lipoids and proteins were concerned in the antilytic and non-specific complement fixation; also, that extraction with ether or chloroform usually diminished the antilytic and complement-fixing powers of a serum, while enteral and parenteral adminstration of lipoids increased the antilytic and complement-fixing powers. Sera extracted with ether were rendered more antilytic, but heating an extracted serum reduced the antilytic titer compared with plain heated serum. It was further concluded "that both the globuin and albumin (filtrate) fractions of normal rabbit and dog sera possess thermostabile antilytic and complement-fixing properties . . . . The antilytic and complement-fixing substances of normal rabbit and dog serum are not dialyzable."—P. B. H.

Studies in Non-Specific Complement Fixation: V. The Effect of Heat on Normal Rabbit and Dog Sera in Relation to Antilytic and Non-Specific Complement Fixation Reations. JOHN A. KOLMER and MARY E. TRIST. (Jour. Infect. Diseases, 1916, **18**, 64–87.)

The authors had already shown 1) the ability of normal rabbit and dog sera to yield non-specific complement fixation with various bacterial and lipoidal antigens; (2) the influence of anesthetics upon this property and (3) the relation of serum lipoids to the process. The aim of the present investigation was to study the influence of certain factors and methods for lessening its effects in complement fixation tests. The tests were conducted with lipoidal extracts and with three bacterial antigens previously mentioned (*vide supra*), the doses being the same as used in Wassermann tests. Guinea pig complement was used. The hemolysins were antisheep (rabbit), antihuman (rabbit) and antiox (rabbit). Tests for the antihemolytic properties of serum were performed by (1) incubating heated serum and complement for one hour; (2) adding the cells and two units of hemolysin; (3) re-incubating for one hour. The complement fixation tests were

conducted by (1) incubating antigen, serum and complement for one hour; (2) adding cells and two units of hemolysin; (3) re-incubating for one hour. The authors conclude from their study as follows: "(1) Non-specific complement fixation by normal rabbit and dog sera is probably due primarily to thermolabil and thermostabil antilytic (anticomplementary) substances in the sera. (2) While fresh and active rabbit and dog sera may yield non-specific complement fixation the tendency is greatly increased as a result of heating the sera. At 56°C. the changes may occur in 20 minutes or even less; at 62°C. for 30 minutes the tendency for non-specific reaction is much decreased and is entirely removed by heating serum at 70°C. for 30 minutes. Changes may occur after exposure at 45°C. for 30 minutes, but the optimal temperature is between 55° and 60°C. (3) In complement fixation tests for specific antibodies with inactivated rabbit, dog and mule sera, it is advisable to heat the sera at 62°C. for one-half hour and to use at least two units of complement or hemolysin and no more than one-quarter of the anticomplementary unit of antigen after it has been carefully titrated. (4) Complementoids and amboceptoids probably bear no relation to the process of non-specific complement fixation by rabbit and dog sera. (5) The blood corpuscles of various animals and various bacteria may absorb a portion of the antilytic substances from rabbit and dog sera, but they have much less influence on the complement fixation reactions. Digestion of fresh sera with corpuscles and bacteria not infrequently increases the anticomplementary properties of the sera. (6) Bacteriolytic amboceptors are not responsible for non-specific complement fixation by normal rabbit and dog sera. (7) Parasitic infestations of rabbits and dogs bear no relation to the antilytic and complement fixing properties of the sera. (8) Single, large doses of salvarsan are without definite influence on the reactions with rabbit serum. (9) Quantitative factors in the hemolytic system and antigen are of considerable importance in relation to these non-specific reactions. (10) If time permits, preliminary complement fixation tests should be performed with the sera of rabbits or dogs before immunization or inoculation is begun, and only those animals selected the sera of which react negatively with the antigen used."—P. B. H.

INDUSTRIAL BACTERIOLOGY

The Importance of Bacterium bulgaricus Groups in Ensilage. O. W. HUNTER and L. D. BUSHNELL. (Science, **43**, 318–320.)

Various kinds of ensilage were examined at different stages of fermentation. On acidulated glucose agar only *Bacterium bulgaricus* and yeasts developed. The colonies of *B. bulgaricus* resembled *Bacterium lactis* and the authors believe that it is on this account that other investigators have overlooked them. It is concluded that the Bulgarian groups occur in sufficiently large numbers, and at a proper stage in ensilage fermentation, to play an important rôle.—C. M. H.

LABORATORY TECHNIQUE

An Eye-Shade for Use with the Microscope. E. KELLERT. (Jour. A. M. A., 1916, **66**, 1023–1024.)

A device for attachment to the draw-tube of the microscope is described. It is so designed as to prevent diffused light from entering the eye above the ocular.—G. H. S.

An Apparatus for Filling Vaccine Ampoules. R. G. Davis, U. S. Naval Med. Bulletin, 1916, **10**, 311–313.

By the use of this apparatus which is briefly described and figured, and which can be made in any laboratory it is claimed that ampoules may be filled with vaccine without loss of time or vaccine.—E. B. V.

The Use of the Sand Tube in Isolating the Bacillus typhosus. M. D. LEVY. (Journ. A. M. A., 1916, **66**, 1022–1023.)

A pipette 33 cm. long and 5 to 6 mm. in diameter is bent in a U shape. Sand is placed in one arm to a height of 10 cm. and the other arm is filled with hot bouillon. The bouillon is inoculated and the tube incubated for 18 hours.

Motile bacilli, such as *Bacillus typhosus* or occasionally *Bacillus coli*, penetrate through the sand and may be isolated from the bouillon above the sand.—G. H. S.

On a Rapid Method of Cultivating the Gonococcus. WM. B. WHERRY and WADE W. OLIVER. (Lancet Clinic, 1916, **115**, 306.)

The authors found that gonococci from the urethral pus of a boy, grew best on Martin's pleuritic agar, under partial oxygen tension. Tubes similarly inoculated and grown aerobically, yielded no growth.

The partial oxygen tension was secured by attaching the culture tubes inoculated with the pus containing gonococci, to similar tubes inoculated with *Bacillus subtilis*. When isolated in this way the gonococci can not be subcultured aerobically, but partial tension subcultures grow promptly.—O. B.

A New Method of Separating Fungi from Protozoa and Bacteria. N. KOPELOFF, H. C. LINT and D. A. COLEMAN. (Bot. Gaz. 1916, **61**, 247–250.)

The dilution method followed by the peculiar manner of plating, makes it possible to separate fungi from bacteria and protozoa.

As the result of this separation it has been possible to eliminate fungi from experiments involving the effect of protozoa on bacterial activity, by making a sub-culture from the fungi-freed solution of bacteria and protozoa.

In view of the fact that fungi are capable of producing ammonia, their presence may introduce a factor not accounted for in measuring the effect of soil protozoa on soil bacteria.—J. T. E.

Study of the Blood with a New Stain. B. LEMCHEN. (Medical Record, 1916, **89**, 607–608.)

The stain consists of a saturated solution of benzidine in absolute alcohol. Blood smears are made on slides and placed in the stain for one-half minute. The slide is then placed in hydrogen peroxide for one-half minute, washed in water and dried on filter paper.

In studying blood stained in this way, it is assumed that cells and tissues of similar composition react in the same way, as staining is a chemical reaction. Red cells, nucleated red cells including both cell and nucleus, and fibrin stain blue; white cells and blood platelets do not take the stain. From this it may be concluded that red cells and white cells are of different origin, that platelets do not have their origin in the nucleus of the red cells, and that fibrin has the same composition as the red cells.

These conclusions may throw some light on the processes of coagulation of the blood and certain phases of hemophilia leading to pernicious anemia. According to this line of reasoning, it may be possible that the origin of agglutinins in typhoid is in the red blood cells.—M. W. C.

A Method of Demonstrating Bacteria in Urine by Means of the Centrifuge. With Some Observations on the Relative Value of Examinations by Culture or Stained Sediment. E. G. CRABTREE. (Surg., Gyn., and Obstet., 1916, **22**, 221–224.)

The method consists in slow centrifugation to remove the heavier sediment, then rapid centrifugation until the urine is clear in order to throw the bacteria out of suspension. C. calls attention to the danger of mistaking smegma for tubercle bacilli, guinea pig inoculation being the final test for infection with tubercle bacilli. The author thinks inconsistent results are obtained because of lack of uniformity in culture media, etc., while organisms such as *B. coli* may overgrow the others. Microscopical examination assists in determining the degree of infection, and predominating organisms, and if there is a mixed infection helps to determine cultural method to be used.

Comment. The author does not mention the use of Petroff's method for direct culturing of tubercle bacilli or the necessity of using the antiformin method where T. B. is suspected and other infection already exists.—C. P. B.

A Rapid Method of Counting Living Bacteria in Milk and Other Richly Seeded Materials. W. D. FROST. (Journ. A. M. A., 1916, **66**, 889–890.)

A detailed account of the procedure is given. The method is essentially as follows:

"One-twentieth cubic centimeter of milk is mixed with standard nutrient agar and spread over a definite area of a sterile glass slide. When the agar is hard, this little plate culture is put in the incubator for about six hours under conditions which prevent evaporation. It is then dried, given a preliminary treatment to prevent the agar from firmly binding

the stain, stained, decolorized and cleared. When this dried and stained plate culture is viewed under the microscope, the little colonies are definitely stained and appear highly colored on a colorless or slightly colored background. These colonies can be readily counted and the number of bacteria per cubic centimeter calculated."—G. H. S.

Counting Bacteria by Means of the Microscope. R. S. BREED and J. D. BREW. (N. Y. State Sta. Tech. Bul. 49, pp. 31, pls. 2, figs. 5.)

This bulletin contains the results of tests of this method which have been made since those published in an earlier bulletin of the station (N. Y. Dept. Agr. Expt. Sta. Bul. 373, pp. 1–38 (1914)). A general description is given of the technique employed in applying this method to milk, the various processes involved being discussed with reference to possible errors. The result of this investigation are indicated by the following quotations from the authors' summary:

"The results obtained from the examination of samples of milk collected in clean test tubes containing preservatives indicate that just as accurate counts of the number of bacteria present can be made from such samples as can be made if the samples are collected in sterile tubes and iced. . . .

"Capillary pipettes have been found to be more satisfactory for the measurement of 0.1 cc. quantities of milk than standardized wire loops.

"Faulty calibration of pipettes has been found to be a serious cause of error. Allowance must be made for the adhesion of a certain quantity of milk to the pipette if accuracy of measurement is to be secured.

"It has been found that sterilization of pipettes is an unnecessary refinement of technique and that a single pipette may be used for making preparations from a long series of samples, provided it is carefully cleaned in glass-cleaning solutions after each day's use and also cleaned by rinsing in fresh, clean tap water after using in each sample and before passing to the next sample. Carelessless in cleaning pipettes causes marked errors in counts.

"Growth of bacteria has been found to take place in the drops of milk as they dry so that it is important that these be prepared either from samples containing preservatives or that the milk be dried quickly. No growth was detected in the dried films even after incubation in a moist, 37°C. incubator for one to four days.

"The claim made by some that bacteria are removed when the fat drops are dissolved by solvents does not seem to have any foundation in fact. The dried milk-solids-not-fat appear to act as a practically perfect fixative, no detectable mechanical loss of bacteria taking place when the fat drops are removed. On the other hand, serious errors in count are introduced where the bacteria are stained in the milk before the dried films are prepared, because in this way the bacteria are not always sufficiently stained to make it possible to detect the full number present. Where the fat drops are left in the films, even though these be spread out so as to be in a very thin layer, they tend to obscure bacteria and so lower the count. . . .

"Microscopical methods of examining dried milk-films are of value for two purposes: (a) They may be used for the rapid examination of milk in order to grade it according to its bacterial quality, both the number and the character of the bacteria present being taken into account. A microscopical examination permits a fairly accurate guess as to the probable plate count which will be secured from a given sample of milk. (b) They are also useful as research methods, the microscopical method being the only known method which permits a count of the number of individual bacteria. Microscopical counts of the number of isolated individual bacteria and compact clumps present in milk give figures which compare well with those obtained where petri plate methods of counting are used."—H. L. L.

MEDICAL BACTERIOLOGY

Foot and Mouth Disease in Man. R. L. SUTTON and A. O'DONNELL. (Journ. A. M. A., 1916, **66**, 947–949.)

Report of a case.—G. H. S.

Early Tuberculosis of the Cervix. T. S. CULLEN. (Surg., Gyn., and Obstet. **22**, 261.)

Tuberculosis of endometrium and cervix. Patient 25 years of age. Condition rare.—C. P. B.

Some Fatal Ear Cases in the Writer's Practice. O. D. STICKNEY, M.D. (Jour. of Ophth., Otol., and Laryngol., **21**, 189–204.)

Eight cases reported. Five had meningitis following otitis. Pneumococci were isolated from spinal fluid in one case, streptococci from another.—C. P. B.

The Choroidal Tubercle in Tuberculous Meningitis. J. F. BREDECK, M.D. (Am. Jr. Ophthalmol., **23**, 1–8.)

Choroidal tubercles may be found in 2 per cent of the cases of tuberculous meningitis if careful, daily search is made.—C. P. B.

The So-Called Primary Tuberculosis of the Conjunctivita and the Conjunctival Tuberculosis of Lupus Patients. K. K. K. LUNDSGAARD. (Am. Jr. of Ophthal., **33**, 54–59.)

Of 48 patients 19 had primary conjunctival tuberculosis; 29 lupus patients had conjunctival tuberculosis. Former believed to be endogenous and the latter ectogenous.—C. P. B.

An Unique Lesion of the Heart in Systemic Blastomycosis. T. B. HURLEY. (Jour. Med. Res., 1916, **33**, 499–502.)

A report of an autopsy of a case of systemic blastomycosis in which the musculature of the heart was extensively involved. This is, according to the author, the second case of its kind to be reported. No cultural studies are reported.—H. W. L.

Practical Points in the Prevention of Asiatic Cholera. ALLEN J. MCLAUGHLIN. (Bost. Med. and Surg. Jour., 1916, **174**, 483.)

The author describes a rapid method of testing for chronic carriers of the cholera vibrio among immigrants. His steps are: inoculating peptone solution, streaking out on agar, and agglutination, with the possible use of Goldberger's enrichment solution. One hundred to 150 stools a day can be tested by one worker.—E. C. L. M.

Diphtheria in Manila. A. P. GOFF. (Journ. A. M. A., 1916, **66**, 941.)

As a result of a small outbreak of virulent diphtheria the Bureau of Science took more than 7000 throat cultures, finding 600 (or 9 per cent) positive for diphtheria.

Of the carriers found, 4 per cent developed symptoms of diphtheria.

G. H. S.

The Etiology and Treatment of Rat-Bite Fever. W. TILESTON. (Journ. A. M. A., 1916, **66**, 995–998.)

A case of rat-bite fever is reported. Organisms were found in the blood by darkfield examination which closely resembled *Streptothrix muris-ratti*. These organisms were to be found only during the febrile paroxysm, examinations made during the intervals being uniformly negative.

The administration of salvarsan was followed by a cessation of the paroxysms.—G. H. S.

Pathogeny of Diabetes and Fecal Disinfection. G. D. PALACIOS. (Medical Record, 1916, **89**, 543–551.)

The pathogeny of diabetes mellitus is a fecal putrefaction and a fecal reabsorption of ammoniacal and acid character. Although fasting and a very restricted diet are the best dietetic treatment of diabetes, fecal disinfection is both preventive and curative.

Intestinal putrefaction may be overcome in some cases by the acidogenous *Bacillus bulgaricus*. In the tropical Atlantic region, an absolute intestinal disinfection is effected by the ingestion of *Micrococcus oxycyanogenes*.—M. W. C.

The Etiology of Scarlet Fever. F. B. MALLORY, and E. M. MEDLAR, (Jour. Med. Res., 1916, **34**, 127–130.)

In a short communication, the authors describe finding, in the crypts of the tonsils, and in erosions of the epithelium of the tonsils, fauces, soft palate, uvula, trachea and lung of a child dying of scarlet fever on the second day following the eruption, clumps of Gram-positive bacilli, together with streptococci. Similar organisms were found in four other cases. The organism is best grown anaerobically on 3 per cent glycerin, 0.5 per cent glucose serum-agar. The authors believe that the organism dies out rapidly or is overgrown by streptococci which accounts for previous failures. In view of the lack of animal experiments

and the small number of cases examined, the work does not appear to be conclusive.—H. W. L.

The Etiology of Rocky Mountain Spotted Fever. S. B. WOLBACH, (Jour. Med. Res., 1916, **34**, 121–127.)

Guinea-pigs inoculated by ticks infected with the virus of spotted fever, show definite pathological changes characteristic of this disease. The author finds in the diseased tissue an organism, agreeing in most respects with that of Ricketts, which he feels justified in calling a bacillus. This organism is described as Gram-negative, resembling somewhat *B. influenzae* but stained bluish by Giemsa, in contrast to most bacteria. All attempts to cultivate the organism have failed.

H. W. L.

Studies on Treponema pallidum and Syphilis. II. Spirochaeticidal Antibodies against Treponema pallidum. H. ZINSSER, and J. G. HOPKINS. (Jour. Exp. Med., 1916, **23**, 323–328.)

Cultures of the *Treponema* were grown on a new medium consisting of inspissated egg in tubes filled with broth serum mixtures. This method makes it possible to obtain clean antigen, unmixed with tissues detritus, a disadvantage incident to tissue cultures. The authors believe that their experiments have shown that the serum of rabbits and sheep immunized with cultures of *Treponema pallidum* acquire spirochaeticidal properties for these culture spirochaetes. The normal serum of these animals also possesses spirochaeticidal action if used in sufficient quantities, and the action of the immune serum represents probably an increase of normal antibodies. Both normal and immune spirochaeticidal properties are destroyed by heating to 56°C. but the serum can be reactivated by the addition of fresh normal serum of the same species, insufficient in amount to exert a spirochaeticidal effect by itself. The structure of these spirochaeticidal bodies appears to be analogous to that of the well-known bactericidal antibodies known to exist in antibacterial sera. It is pointed out by the authors that these results apply to culture spirochaetes.—G. B. W.

III. The Individual Fluctuations in Virulence and Comparative Virulence of Treponema pallidum Strains Passed Through Rabbits. HANS ZINSSER, J. G. HOPKINS, and M. MCBURNEY. (Jour. Exp. Med., 1916, **23**, 329–340.)

Rabbits were inoculated with strains from human cases with the purpose of studying differences in racial and acquired virulence. The authors found no difference in pathogenicity between the different strains, although they were isolated from various lesions, and, further, these strains show no consistent change in rabbit pathogenicity during progressive rabbit passage (21 generations in one case). Variations in the lesions produced, and also in the incubation time are probably due to variations in technique.—G. B. W.

IV. The Difference in Behavior in Immune Serum between Cultivated Non-Virulent Treponema pallidum and Virulent Treponemata from Lesions. HANS ZINSSER, J. G. HOPKINS, and M. MCBURNEY. (Jour. Exp. Med., 1916, **23**, 341–352.)

Although antibodies can be produced by the immunization of animals with cultivated *Treponema pallidum*, and although these antibodies exert specific agglutinative and treponemicidal action upon the culture organisms, they possess, at least in the concentration so far obtained by the authors in rabbits and sheep, practically no action on virulent treponemata obtained directly from lesions.—G. B. W.

An Experimental Study of Parotitis (Mumps). MARTHA WOLLSTEIN. (Jour. Exp. Med., 1916, **23**, 353–375.)

Cats injected in the parotid gland and testicle with a bacterial sterile filtrate of the salivary secretion of children in the active stage of parotitis, or mumps, can be made to develop a pathological condition having several points of resemblance to the condition present in mumps in human beings. Definite changes in the temperature, blood leucocytes, and inoculated organs take place after an incubation stage of from 5 to 8 days. These pathological changes are intensified by successive transfers through a small series of cats of the extract and emulsion of the parotid gland and testicle previously inoculated. These changes can also be prevented or reduced when the extract or emulsion is previously incubated with blood serum obtained from a cat which has survived inoculation. Normal serum, on the other hand has no such inhibiting effect. Whether the filtered salivary secretion contains a microörganism and, if so, whether it is the specific microbic cause of parotitis, or mumps, remains to be ascertained.

G. B. W.

The Etiology, Mode of Infection, and Specific Therapy of Weil's Disease (Spirochaetosis icterohaemorrhagica). R. INDA, Y. IDO, R. HOKI, R. KANEKO and H. ITO. (Jour. Exp. Med., 1916, **23**, 377–402.)

In the course of their investigations of that endemic disease of portions of Japan, which agrees clinically with Weil's disease, so called, the authors discovered a spirochaetal microörganism which they name *Spirochaeta icterohaemorrhagiae*, and which they believe to be the cause of the disease. These spirochaetes live in the blood outside the cellular elements and in various organs and tissues. Infection is supposed to be by way of the alimentary canal or it may enter through the skin. The spirochaetes are excreted through the urine. The serum of convalescents possesses bactericidal and bacteriolytic properties and recovery from the disease confers a lasting immunity. Treatment with salvarsan appears to offer promising possibilities, while passive immunization with immune serum has already given gratifying results. Many excellent plates are appended.—G. B. W.

Bacteria Associated with Certain Types of Abnormal Lymph Glands. J. C. TORREY. (Jour. Med. Res., 1916, **34**, 65–81.)

With a view to substantiating the claims of various authors that a diphtheroid bacillus is the causative agent in Hodgkin's disease, the author cultured 40 abnormal lymph glands, including 10 cases of Hodgkins. Three distinct groups of aerobic diphtheroid bacilli, one anaerobic group, and various other types of organisms were found and such a diversity of pathological conditions as to preclude the possibility of attaching importance to any one type as the cause of Hodgkin's disease. Serological reactions and animal inoculations failed to show any specificity.

The finding of the anaerobic diphtheroid type in 100 per cent of the cases of Hodgkin's as well as in various other conditions, although interesting, the author does not believe is of any importance as throwing light on the cause of the disease in question, and only emphasizes the need of caution in accepting uncontrolled results as conclusive evidence.—H. W. L.

The Diagnosis of Genitourinary Tuberculosis. J. W. CHURCHMAN. (Medical Record, 1916, **89**, 511–513.)

The mode of entrance of tubercle bacilli into the urine is not definitely proved, but it seems probable that the normal kidney is permeable for the tubercle bacillus as well as for other organisms. That infection does not extend upward from bladder to kidney is a well-established fact.

The most reliable sign in diagnosing renal tuberculosis is the presence of tubercle bacilli in the urine, though in rare cases there may be tuberculosis of the kidney with no demonstrable tubercle bacilli in the urine. In such cases the granules described by Much may be worthy of attention. These granules are interpreted by him to represent types of the tubercle bacillus which do not possess the ordinary acid-fast character. Although it is impossible to say what the significance of the Much granules is, it is true that they were present in the urine in a case of tuberculous kidney where tubercle bacilli were not found in the urine.—M. W. C.

Relapsing Fever in Serbia. J. RUDIS-JICINSKY. (New York Med. Jour., 1916, **103**, 643–645.)

Hundreds of cases of relapsing fever occurred in Serbia during the past winter.

In many cases examination of the blood revealed *Spirillum obermeieri.* This organism was filamentous, of spiral form, much elongated, and in motion followed its long axis. It was about four times the diameter of a red blood corpuscle. The organism was aerobic. It could be stained easily with anilin colors in dry blood, but was not found in other fluids or secretions of the body.

The stage of the disease at which the spirochaete could be found in the blood was not always the same.

Inoculation of blood containing spirochaetes into rabbits conveyed the disease to these animals. After death spirochaetes were present in all the organs, but could not be cultivated upon artificial media.

In nearly every case, the louse could be considered as the carrier of the infection, and the prevention of lousiness was a necessary step in eradicating the disease.—M. W. C.

Colon Bacillus Infection of the Bladder. R. T. MORRIS. (New York Med. Jour., 1916, **103**, 631–632.)

Many cases of cystitis of obscure origin may be due to the colon bacillus, as this bacillus is sometimes found upon examination of the urine, and conditions similar to cystitis have been induced in experimental animals by injecting *Bacillus coli* into the bladder.

The differences in the type of infection are probably due to the particular strain of colon bacillus causing the condition, for there is a wide variation among the members of this group. According to the recent work of Rosenow it may even be possible that unusual types of cystitis are caused by other bacilli, which have assumed the form of *B. coli*. Uncertain action of vaccines in cases of colon bacillus infection is perhaps due to such a variation in the infecting organism.

Colon bacilli may be responsible for any of the widely different manifestations of cystitis.

The mode of entrance of the colon bacillus into the bladder probably differs with varying conditions.—M. W. C.

Studies on Diphtheria. II. The Treatment of Diphtheria Carriers by Tonsillectomy. H. O. RUH, M. J. MILER and R. G. PERKINS. (Journ. A. M. A., 1916, **66**, 941–943.)

The termination of the carrier condition through the use of biological or chemical methods did not meet with great success.

In a series of 19 cases tonsillectomy was performed. The average duration of the carrier state before operative treatment was resorted to was 31 days. The average duration of release from quarantine after the tonsillectomy was 8 days.

In all cases cultures made from the crypts after excision yielded *Bacillus diphtheriae* in nearly pure culture although surface cultures were frequently negative.—G. H. S.

Bacteriological Work at the American Ambulance. ORVILLE F. ROGERS and GEORGE BENET. (Bost. Med. and Surg. Jour., 1916, **174**, 418.)

The authors report on the bacteriological work done in the Harvard University Service of the American Ambulance from April 1 to July 1, 1915.

From 100 men examined 28 showed gas-producing organisms. Of these 28, 18 were obtained in pure culture and run through sugar media. The authors conclude that sugar media are not suitable for the differentiation of the gas-producing bacilli.

"The majority of cultures showed staphylococci either alone (30)

or with other organisms (58). Forty cultures showed an anaerobic growth of other than gas producers. Other organisms seen were: pneumococcus (11), streptococcus (9), pyocyaneus (5), and varieties of other than gas producers (25)."—E. C. L. M.

The Use of Kaolin to Remove Diphtheria Bacilli from the Nose and Throat. B. RAPPAPORT. (Journ. A. M. A., 1916, **66**, 943–945.)

A study of the use of kaolin in 100 cases, 96 being diphtheria patients and 4 carriers.

Kaolin, thoroughly dried and finely powered, is distributed over the surfaces to be treated. In young children application can best be made to the nasal mucous membrane even though the bacilli are in the pharynx. Some of the kaolin will work its way into the throat but the greater part will remain in the nose. Before a second treatment the kaolin already applied and now holding organisms should be removed by a mild alkaline spray.

Six treatments per day at two hour intervals are given. With older patients the kaolin is swallowed, four half teaspoonful doses at two hour intervals six times during the day.

The action of kaolin appears to be wholly mechanical, no bactericidal action being evident.

The nose may be freed of bacilli much more readily than the throat.

Compared with 100 consecutive cases dismissed before the use of kaolin, the treatment effected a percentage reduction of hospital stay of 23.4.

Various pathological conditions, as adenoids and diseased tonsils, interfere with the action of kaolin. In such cases surgical treatment is required.—G. H. S.

The Practical Value of Guinea Pig Tests for the Virulence of Diphtheria Bacilli. JOHH A. KOLMER, SAMUEL S. WOODY. EMILY L. MOSHAGE. (American Jour. Diseases of Children, 1916, **4**, 257–268.)

The paper is based upon the results obtained with the guinea pig test for virulence on 1054 diphtheria cultures. The method employed consists of isolation of the bacilli upon slants of Loeffler's blood serum media, subculturing in 0.2 per cent glucose broth with a reaction of plus 0.8, incubation at 37°C., for seventy-two hours and injection subcutaneously in the median line of a pig weighing from 250 to 300 grams with a dose corresponding to 0.5 per cent of the weight of the animal expressed in cubic centimeters. The total amount injected is brought up to 4 cc. The animal is observed for four days and the development of a typical local inflammation with toxemia is regarded as diagnostic If in doubt a second pig is inoculated and at the same time is given 500 units of diphtheria antitoxin. 4 cc. of a good 24 hour culture grown upon a tube of Loeffler's blood serum washed off in 10 cc. of salt solution can also be used for injection and has the advantage of saving 48 hours in time. Granular and barred types were found virulent in about 70 per cent of cultures from throat, nose and ear, long

solid forms in about 42 per cent of cultures, while short solid types were uniformly found to possess no virulence. The authors, especially emphasize the importance of tests for virulence in recovery cases before dismissal and in suspected carrier cases.—R. M. T.

A Preliminary Report on Pneumonia in Children, with Special Reference to its Epidemiology. GODFREY R. PISEK and MARSHALL C. PEASE. Am. Jour. Med. Sc., 1916, **151**, 14.

In an analysis of 1000 cases of pneumonia, not including those cases secondary to other infectious diseases, the authors found a mortality of 34.5 per cent in children under six years of age in the Babies' Wards of the New York Post-Graduate Hospital. The series contained 445 cases classed as bronchopneumonia which occurred chiefly during the first two years of life, and were relatively uncommon after the third year. The lobar form also occurred more frequently during the first two years, and was the type usually found after the third year if the terminal and secondary infections following other diseases are excluded. The highest mortality was found in the first year of life with both forms, but relatively less frequently with the lobar. No evidence was found of either epidemic or house infection in studying the cases. The authors felt that the broncho- and lobar types formed rather distinctive groups clinically, their conclusions being based upon both pathological and bacteriological differences. For bacteriological study the materials were taken from the upper part of the larynx by means of a bent applicator. In 23 cases of lobar pneumonia, with sputum virulent for mice, all showed Gram-positive diplococci predominating in nearly every case with a few streptococci and staphylococci. In 10 cases of mild bronchopneumonia with sputum which seemed virulent to mice, the predominating organism in 5 cases was the streptococcus. 2 cases each showed staphylococci and the influenza bacillus, and in 1 case, tubercle bacilli with other organisms. In 4 cases a few pneumococci were present. In 8 cases of bronchopneumonia with sputum virulent to mice, smears showed large numbers of pneumococci, in addition to large numbers of other organisms, chiefly streptococci and staphylococci. Bacteriologically, the authors consider bronchopneumonia as being a mixed infection, or an infection chiefly with one type of organism other than the pneumococcus. This differentiates this form from the lobar type, which is due chiefly or entirely to the pneumococcus. A study was made of the types of pneumococci occurring in a group of 48 clinical cases of pneumonia, which gave conclusive results. Of these, 28 cases were classified as lobar and 20 cases as bronchopneumonia. For this purpose the Dochez-Gillespie grouping of pneumococci was followed, using specific sera for Groups I and II prepared by the Rockefeller Institute, and the methods recommended by them. The series gave the following results: Group I, 11 cases; Group II, 14 cases; Group III, 4 cases; and Group IV, 19 cases. Cases clinically classed as lobar pneumonia showed pneumococci Groups I and II, decidedly predominating, while more than

half of the clinical bronchopneumonias fell in Group IV. The mortality rate according to groups was as follows: Group I, 9 per cent.; Group II, 36 per cent.; Group III, 25 per cent.; and Group IV, 21 per cent. Eleven strains of pneumococci taken from the throats of children showing no lung involvement fell in Group IV.—L. W. F.

PALEONTOLOGY

Mesozoic Pathology and Bacteriology. Roy L. Moodie. (Science, 1916, **43**, 425–426.)

Attention is called to this rather unusual, though fascinating and important branch of bacteriology. The author feels that we have convincing proof of the existence of fungi and bacteria in coprolites, and of pathologic conditions in various fossil tumors and fractures. —C. M. H.

PLANT PATHOLOGY

Further Studies in the Rôle of Insects in the Dissemination of Fire Blight Bacteria. V. B. Stewart and M. D. Leonard. (Phytop. 1916, 152–158.)

From observations made throughout several seasons the writers believe that all the sucking bugs of the nursery bear infection. Experiments were conducted by caging upon trees insects which had on their bodies organisms from a pure culture of *Bacillus amylovorus*. The various species of flies are thought not to be active agents in transmiting infection though they may be important in carrying the organism to blossoms or to wounds. The experiments included the following suspected carriers: *Pollenia rudis, Empoasca mali, Psylla pyricola, Plagiognathus politus, Sapromyza bispina.*—F. L. S.

Citrus Canker. F. A. Wolf. (J. Agr. Res. **6**, 69–99.)

A serious citrus disease has recently been introduced into the Gulf States, known as citrus canker. The primary cause is *Ps. citri* Hasse, an organism with a single flagellum, shown by the writer to have the group number Ps. 221. 3332513. It attacks both twigs and leaves. Fungi of the genera *Phoma, Fusarium* and *Gleosporium* have been found associated with this organism, although the *Phoma* is the only one found to be notably active in disintegration of the tissues. The only method of control recommended is by means of quarantine and thorough destruction of diseased trees.—H. J. C.